# BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

## IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images, please do not report the images to the Problem Image Mailbox.**

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 47/48, 49/00, G01N 33/50

A2

(11) International Publication Number: WO 99/24076

(43) International Publication Date: 20 May 1999 (20.05.99)

(21) International Application Number: PCT/US98/23706

(22) International Filing Date: 6 November 1998 (06.11.98)

(30) Priority Data:
60/064,705 7 November 1997 (07.11.97) US

(71) Applicant (*for all designated States except US*): CONJUCHEM, INC. [CA/CA]; Suite 810, 1801 boulevard de Maisonneuve West, Montreal, Quebec H3H 1J9 (CA).

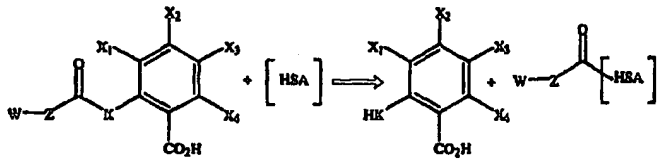
(72) Inventors; and
(75) Inventors/Applicants (*for US only*): KRANTZ, Alexander [US/US]; 847 North Humboldt Street #310, San Mateo, CA 94401 (US). SONG, Yong Hong [CA/US]; 1144 Nimitz Lane, Foster City, CA 94404 (US). BRIDON, Dominique, P. [FR/CA]; 243 chemin Cote Ste-Catherine, Outremont, Quebec H2V 2B2 (CA). HARDY, Margaret [-/CA]; Conjuchem, Inc., Suite 810, 1801 boulevard de Maisonneuve West, Montreal, Quebec H3H 1J9 (CA). CHEN, Qui Qui [-/CA]; Conjuchem, Inc., Suite 810, 1801 boulevard de Maisonneuve West, Montreal, Quebec H3H 1J9 (CA). SETTINERI, Tina [-/CA]; 850 Pointe Pacifica #1, Daly City, CA 94014 (US).

(74) Agents: WARD, Michael, R. et al.; Limbach & Limbach L.L.P., 2001 Ferry Building, San Francisco, CA 94111-4262 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published**
*Without international search report and to be republished upon receipt of that report.*

(54) Title: SALICYLATE DERIVATIZED THERAPEUTIC AND DIAGNOSTIC AGENTS




(57) Abstract

The present invention relates to novel salicylate compounds which comprise a salicylate moiety and a biologically active agent of interest, where the compound binds and bonds specifically through reaction of the salicylate ester to serum albumin at Lys 199. Albumin is a long-lived moiety present in the blood. Novel salicylate compounds of the present invention provide for long-lived maintenance of the biologically active entity in the host. These compounds find broad applications in prophylactic and therapeutic applications, production of antibodies, reduction of the biologically effective concentration or activity of an endogenous or exogenous blood component, and in other situations where long term administration of a physiologically active compound is of interest.

AM2

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **AL** | Albania | **ES** | Spain | **LS** | Lesotho | **SI** | Slovenia |
| **AM** | Armenia | **FI** | Finland | **LT** | Lithuania | **SK** | Slovakia |
| **AT** | Austria | **FR** | France | **LU** | Luxembourg | **SN** | Senegal |
| **AU** | Australia | **GA** | Gabon | **LV** | Latvia | **SZ** | Swaziland |
| **AZ** | Azerbaijan | **GB** | United Kingdom | **MC** | Monaco | **TD** | Chad |
| **BA** | Bosnia and Herzegovina | **GE** | Georgia | **MD** | Republic of Moldova | **TG** | Togo |
| **BB** | Barbados | **GH** | Ghana | **MG** | Madagascar | **TJ** | Tajikistan |
| **BE** | Belgium | **GN** | Guinea | **MK** | The former Yugoslav Republic of Macedonia | **TM** | Turkmenistan |
| **BF** | Burkina Faso | **GR** | Greece | | | **TR** | Turkey |
| **BG** | Bulgaria | **HU** | Hungary | **ML** | Mali | **TT** | Trinidad and Tobago |
| **BJ** | Benin | **IE** | Ireland | **MN** | Mongolia | **UA** | Ukraine |
| **BR** | Brazil | **IL** | Israel | **MR** | Mauritania | **UG** | Uganda |
| **BY** | Belarus | **IS** | Iceland | **MW** | Malawi | **US** | United States of America |
| **CA** | Canada | **IT** | Italy | **MX** | Mexico | **UZ** | Uzbekistan |
| **CF** | Central African Republic | **JP** | Japan | **NE** | Niger | **VN** | Viet Nam |
| **CG** | Congo | **KE** | Kenya | **NL** | Netherlands | **YU** | Yugoslavia |
| **CH** | Switzerland | **KG** | Kyrgyzstan | **NO** | Norway | **ZW** | Zimbabwe |
| **CI** | Côte d'Ivoire | **KP** | Democratic People's Republic of Korea | **NZ** | New Zealand | | |
| **CM** | Cameroon | | | **PL** | Poland | | |
| **CN** | China | **KR** | Republic of Korea | **PT** | Portugal | | |
| **CU** | Cuba | **KZ** | Kazakstan | **RO** | Romania | | |
| **CZ** | Czech Republic | **LC** | Saint Lucia | **RU** | Russian Federation | | |
| **DE** | Germany | **LI** | Liechtenstein | **SD** | Sudan | | |
| **DK** | Denmark | **LK** | Sri Lanka | **SE** | Sweden | | |
| **EE** | Estonia | **LR** | Liberia | **SG** | Singapore | | |

# SALICYLATE DERIVATIZED THERAPEUTIC AND DIAGNOSTIC AGENTS

## RELATED APPLICATION

This application claims the benefit under 35 U.S.C. § 119(e) of United States Provisional Patent Application number 60/064,705 filed November 7, 1997.

## FIELD OF THE INVENTION

The invention relates to salicylate compounds for the specific covalent labeling of serum albumin at Lys 199 in a host and allows for the controlled distribution and the extended lifetime of the therapeutic or the diagnostic agent in the host.

## BACKGROUND OF THE INVENTION

In many situations, there is a need for long term administration of a drug, so as to maintain a therapeutic level in the blood stream. The oral administration or intravenous injection of drugs can result in subtherapeutic dosages for extended periods of time, followed by dosages exceeding the therapeutic level and involving serious side effects. Also, there are situations where one wishes to maintain an agent in the blood stream for an extended period of time, for example, when one wishes to obtain sustained duration of action when performing diagnostic imaging or for long lasting therapy.

There is, therefore, substantial interest in being able to improve methods for delivering agents to the blood with sustained duration of action, where the function of the agent may be for limiting the toxicity or pathogenicity of blood-borne agents, long term action of a physiologically active agent, or long term delivery of an active agent.

Delivery of therapeutic agents to a mammalian host can frequently be as important as the activity of the drug in providing effective treatment. For the most part, drugs are delivered orally, frequently initially at a dosage below the therapeutic dosage and by repetitive administration of the drug, the dosage is raised to a therapeutic level or a level exceeding the therapeutic level. In many cases, the fact of having a dosage above therapeutic level provides for adverse effects, since most drugs are not only effective for the intended purpose, but frequently have adverse side effects. Various proposals have been made to avoid these problems, such as slow-release capsules, depots, pumps, and the like. These various approaches have numerous short comings for general applications where one wishes to maintain the presence of a therapeutic agent at a therapeutic dosage for an extended period. Invasive procedures are frequently undesirable, requiring surgery for introduction of the delivery device, followed by subsequent removal. The delivery device is placed on the skin from which the agent must be capable of transport across the skin at the desired rate. Slow-release particles have a limited time span and when introduced into the blood stream will be rapidly phagocytosed.

For those therapeutic and diagnostic agents which must be administered by injection, the need to have repetitive injections is particularly undesirable. The need in many cases for self administration is particularly problematic and in many instances may require trained individuals for the administration. There is, therefore, a serious need for compositions and methods which would allow for extended administration of therapeutic and diagnostic agents, particularly in the blood stream, which can be easily administered while its efficacy maintained for extended period of time.

Serum albumin is the most abundant plasma protein in blood stream. Specifically and covalently linking drug molecules or diagnostic

agents to serum albumin and using the resulting drug-albumin or diagnostic agent-albumin conjugates as therapeutic agents are attractive ways to extend the duration of action of the drug or diagnostic agents, because albumin has a long lifetime in the blood stream (days to weeks). However, previous methods of bioconjugation involving albumin have employed non-specific reactive groups, such as N-hydroxysuccinimide ester (NHS), which react indiscriminately with different residues on various proteins. As a result, a heterogeneous mixture of bioconjugates is usually produced. Although some reactive groups can selectively react with one type of residue (such as maleimide with the free thiol of cystein), they are not easily suitable for conjugating drugs to serum albumin *in vivo* because they will indiscriminately react with the same type of residue present on other proteins in the body (other free thiol on other proteins as described in E Schauenstein et al., 1986)).

Salicylates (e.g. aspirin) have been successfully utilized to specifically cross-link hemoglobin tetramer in previous endeavor of developing hemoglobin-based blood substitutes and antisickling agents. In previous practice, salicylates themselves are the agents which directly interact with disease targets. Salicylates are well-documented and widely used drug molecules. Acetylsalicylate is known to specifically acetylate human serum albumin at Lys 199. However, it is not known how one might utilize salicylate to deliver therapeutic and diagnostic molecules to human serum albumin for specific labeling of albumin.

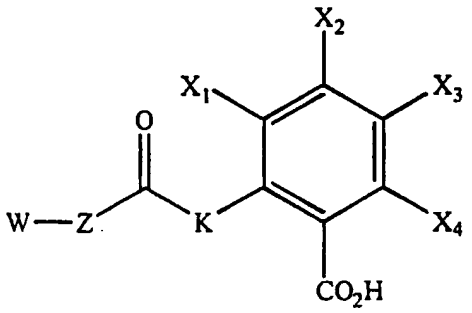
It would be highly desirable to use a salicylate compound for specific covalent labeling of serum albumin with therapeutic and diagnostic agents for the purpose of obtaining a sustained duration of action and a controlled distribution.

## SUMMARY OF THE INVENTION

One aim of the present invention is to deliver a salicylate compound for specific covalent labeling of serum albumin with therapeutic and diagnostic agents, which allows for the extended lifetime of the therapeutic

and diagnostic agents. In a preferred embodiment of the present invention, a salicylic acid moiety is released upon covalent attachment of the therapeutic or diagnostic agent to serum albumin.

In accordance with one embodiment of the present invention, there is provided a salicylate compound for specific covalent labeling of albumin at Lys 199, which comprises a compound of the formula:



wherein:

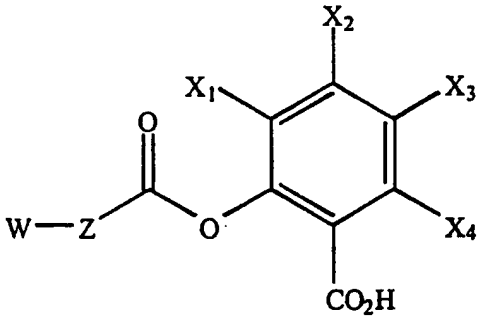
$X_1$, $X_2$, $X_3$ and $X_4$ are H, Cl, Br, I, F, $NO_2$, CN, $MeCO_2$ or MeO, with the proviso that $X_1$, $X_2$, $X_3$ and $X_4$ are different or the same;

Z is a spacing group which links W and wherein the linked W remains bioavailable and bioactive;

W is a therapeutic or diagnostic agent; and

K is Oxygen (O) Sulfur (S) or Nitrogen (N)

In accordance with another embodiment of the present invention, there is provided a salicylate compound for specific covalent labeling of albumin at Lys 199, which comprises a compound of the formula:



wherein:

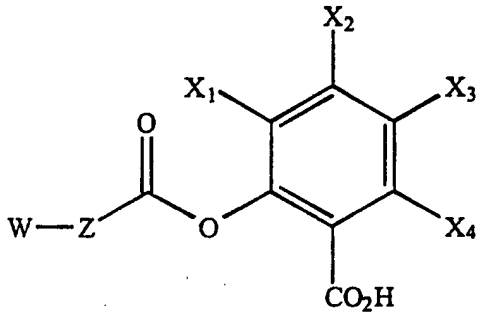
$X_2$ and $X_4$ are H;

$X_1$ and $X_3$ are Cl, Br, I, F, $NO_2$, CN, Me or MeO, with the proviso that $X_1$ and $X_3$ are different or the same;

Z is a spacing group which links W and wherein the linked W remains bioavailable and bioactive; and

W is a therapeutic or diagnostic agent.

In accordance with another embodiment of the present invention, there is provided a salicylate compound for specific covalent labeling of albumin at Lys 199, which comprises a compound of the formula:




wherein:

$X_1$, $X_2$ and $X_4$ are H;

$X_3$ is Br;

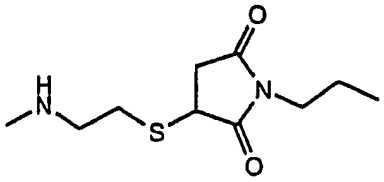
Z is a spacing group which links W and wherein the linked W remains bioavailable and bioactive; and

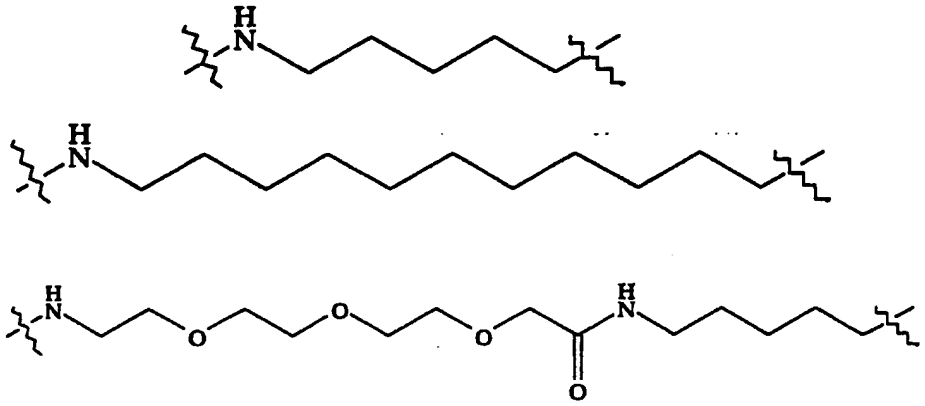
W is a therapeutic or diagnostic agent.

Preferably, the Z group may be of at least 6 atoms. More preferably, Z includes, without limitation, alkyl, alkoxy, alkenyl, alkynyl, amino, alkyl-substituted amino groups, cycloalkyl, polycyclic, aryl, polyaryl, substituted aryl heterocyclic and substituted heterocyclic groups including 1,4-disubstituted benzenes or cyclohexanes.

The preferred Z group is a Z moiety having any of the following formula:

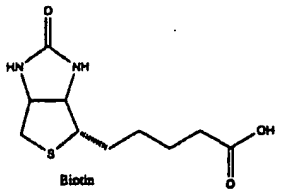
$—NH(CH_2)_n—$ where n=5,11



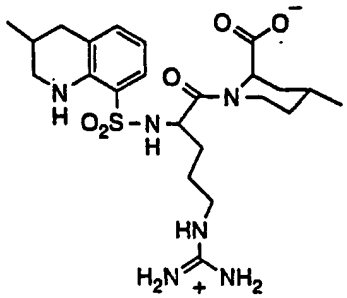


Preferably, the W group is a therapeutic or diagnostic agent. More preferably, the W group would include, without limitation, any drug or small molecule of molecular weight less than 2000 to be administered to a patient in need of such agent. A wide variety of hormones, cytokines, surface membrane protein receptors, synthetic drugs, enzyme inhibitors, cytotoxic compounds, antibodies, T-cell receptors, active peptides, homing receptors, prostaglandins, catalytic antibodies, or the like, or physiologically active fragments thereof may be conjugated to the salicylic acid moiety of this invention. Illustrative compounds may include growth factors, e.g. colony stimulating factors, including M-, G-, G, M-; interleukins 1-10, interferons, epidermal growth factor, fibroblast growth factor, platelet-derived growth factor, transforming growth factor-α, or -β, neuroactive peptides, RGD peptides, dynorphin peptides, opioid peptides, renin inhibitors, calcium channel blockers, tumor necrosis factor, etc., or corresponding cell receptors; insulin, somatomedin, somatostatin, follicular growth hormone, ACTH, VIP, antibiotics such as penicillin, cephalosporin, neomycin, aminoglycosides, quinolones, sulfamides, tetracyclines, sulfones, quinine; antibodies to interleukins, TNF, rheumatoid factors, etc.; vitamins, anti-coagulants, or the like.

The W group may be biotin and have the following formula:




The W group may be argatroban and have the following formula:



The W group may be an opioid molecule. Endogenous opioids exist in multiple forms in the central nervous system, and include the dynorphins, which are a series of peptides derived from the precursor prodynorphin (proenkephalin B). The first of the dynorphins to be isolated was the 17 amino acid peptide having the structure shown (and designated SEQ ID NO:1), sometimes also referred to as "dynorphin A-(1-17)":

Y G G F L R R I R P K L K W D N Q (SEQ ID NO:1)

Various dynorphin analogue compounds including "dynorphin A-(1-13) with the structure:

Y G G F L R R I R P K L K (SEQ ID NO:2)

The W group may be an RGD-containing or a KGD-containing peptide. RGD-containing peptides are known to be potent platelet glycoprotein IIb-IIIa antagonists. Barbourin is a KGD peptide with a reported IC 50 of 300 nM for the inhibition of ADP-induced human platelet aggregation. This peptide has a high affinity for the IIb/IIIA receptor. Cyclic RGD peptides are also included within the scope of the invention.

RGD-containing peptides include the triflavin-like peptide R I A R G D F P D D R K (SEQ ID NO:3).

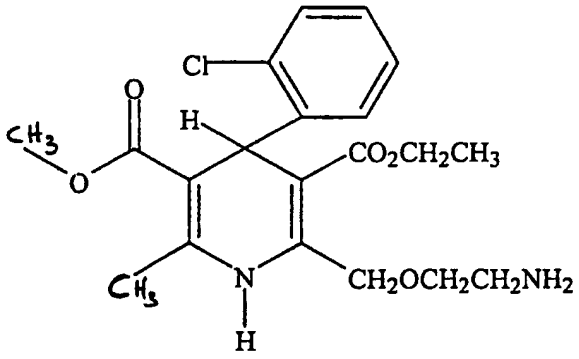
KGD-containing peptides include Barbourin:
C R V A K G D W N D D T C (SEQ ID NO:4).

Cyclic RGD peptides include sequences such as C R I A R G D F P D D R C (SEQ ID NO:5).

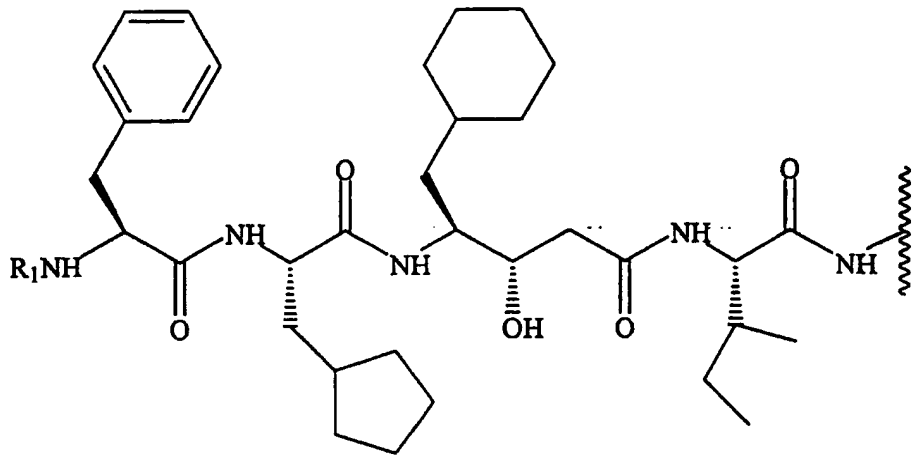
The W group may be a diagnostic agent. Diagnostic agents include agents used in diagnostic techniques such as positron emission tomography (PET), computerized tomography (CT), single photon emission computerized tomography (SPECT), magnetic resonance imaging (MRI), nuclear magnetic imaging (NMI), fluoroscopy and ultrasound, fluorophores, chromophors and chemiluminophors.

Diagnostic agents of interest include contrast agents, radioisotopes of such elements as iodine (I), including $^{123}I$, $^{125}I$, $^{131}I$, etc., barium (Ba), gadolinium (Gd), technetium (Tc), including $^{99}Tc$, phosphorus (P), including $^{31}P$, iron (Fe), manganese (Mn), thallium (Tl), chromium (Cr), including $^{51}Cr$, carbon (C), including $^{11}C$, or the like, fluorescently labeled compounds, and fluorine containing chemicals such as perfluoropropane, perfluorobutane, and perfluoropentane useful in ultrasound, etc.

The W group may be a calcium channel blocker such as an amplodipine shown by the following structure:




The W group may be a renin inhibitor. The derivatized renin inhibitor compounds of the present invention will, for the most part, have the following formula:




wherein $R_1$ is an acyl group of from 1-10 carbon atoms, more usually of from 1-8 carbon atoms, preferably of from about 2-6 carbon atoms, and includes alkoxy carbonyl, where the alkyl group is of 1-5 carbon atoms, more usually of from 2-4 carbon atoms, particularly t-butyloxycarbonyl, formyl, acetyl, propionyl, butyryl, methoxyacetyl, pivaloyl, and the like;

In accordance with the present invention, there is also provided a method for the *in vivo* specific labeling of albumin at Lys 199 with a biologically active molecule, which comprises:

introducing into the bloodstream of a mammalian host a salicylate compound in an amount sufficient to provide an effective amount of a diagnostic or therapeutic agent; wherein the compound reacts with and becomes covalently and specifically bound to serum albumin at Lys 199 and the biologically active molecule remains bioavailable and bioactive while covalently bonded to serum albumin over an extended period of time as compared to the lifetime of unbound biologically active molecule.

The introduction step may be effected intravascularly.

In accordance with the present invention, there is also provided a blood portion comprising a compound of the present invention covalently and specifically bound to serum albumin at Lys 199.

In accordance with the present invention, there is also provided a conjugate comprising a compound of the present invention covalently bound to serum albumin at Lys 199.

In accordance with the present invention, there is also provided a method for the *in vitro* specific labeling of albumin at Lys 199 with a diagnostic or therapeutic agent, which comprises:

introducing into an *in vitro* solution selected from the group consisting of blood, plasma, serum, and serum albumin in saline buffer, a salicylate compound in an amount sufficient to provide an effective amount of the diagnostic or therapeutic agent; wherein the compound reacts with and becomes covalently and specifically bound to serum albumin at Lys 199 to form a diagnostic or therapeutic-albumin conjugate simultaneously with the release of salicylic acid, wherein the albumin conjugate is suitable for injection into the bloodstream of a mammalian host and the therapeutic or diagnostic agent remains bioavailable and bioactive over an extended period of time as compared to the lifetime of unbound biologically active molecule.

The preferred *in vitro* solution is plasma or serum albumin in buffer.

The salicylate compounds of the present invention find broad applications in prophylactic and therapeutic applications, production of antibodies, reduction of the biologically effective concentration or activity of an endogenous or exogenous blood component, and in other situations where long term duration of a physiologically active compound is of interest.

The compounds of the present invention may be administered *in vivo* or *ex vivo*.

## BRIEF DESCRIPTION OF THE DRAWINGS

This invention will be better understood by reference to the figures, in which:

Fig.1 shows a reaction scheme for the use of a preferred embodiment of the invention for covalently attaching a therapeutic and a diagnostic agent to human serum albumin (HSA);

Fig. 2 illustrates the synthesis scheme of Arg-C6 5-bromo salicylic acid (YS-41-13);

Figs. 3A and 3B illustrate the synthesis scheme of Arg-C18 PE salicylic acid (YS-41-94);

Fig. 4 illustrates the synthesis scheme of Biotin salicylates and tBu-salicylates;

Figs. 5A and 5B illustrate the synthesis scheme of a renin inhibitor salicylate derivative;

Fig. 6 illustrates the synthesis scheme of a calcium channel blocker salicylate derivative;

Fig. 7 illustrates the structure of biotin salicylates of one embodiment of the present invention;

Fig. 8 illustrates the structure of argatroban salicylates of one embodiment of the present invention;

Fig. 9 illustrates that the argatroban-albumin conjugate of the present invention can inhibit its target enzyme, thrombin;

Fig. 10 illustrates a Western blot of specific labeling of albumin with biotin-salicylate compound of the present invention when compared to non specific agent NHS biotin and maleimide biotin;

Figs. 11A-11D illustrate tryptic digestion and mass spectrometric analysis of albumin specifically labeled at Lys 199; and

## BRIEF DESCRIPTION OF THE SEQUENCE LISTING

SEQ ID NO:1 is a dynorphin analogue A (1-17).

SEQ ID NO:2 is a dynorphin analogue A (1-13).

SEQ ID NO:3 is an RGD peptide.

SEQ ID NO:4 is a KGD peptide.

SEQ ID NO:5 is an cyclic RGD peptide.

## DETAILED DESCRIPTION OF THE INVENTION

### DEFINITIONS

To ensure a complete understanding of the invention the following

definitions are provided:

**Therapeutic Agents**: Therapeutic agents are agents that have a therapeutic effect. Therapeutic agents include drugs, antibiotics, antiinfectives, anti-cancer agents, pain management agents, cardiovascular drugs (antithrombotics, anticoagulants, anti platelet, protease inhibitors, calcium channel blockers, etc.), anti-inflammatory agents, antiproliferative drugs, oligonucleotides (sense and antisense). Therapeutic agents are generally drugs or small molecules having a molecular weight less than 2000.

**Diagnostic Agents**: Diagnostic agents include agents used in diagnostic techniques such as positron emission tomography (PET), computerized tomography (CT), single photon emission computerized tomography (SPECT), magnetic resonance imaging (MRI), nuclear magnetic imaging (NMI), fluoroscopy and ultrasound, fluorphors, chromophors and chemiluminophors.

Diagnostic agents of interest include contrast agents, radioisotopes of such elements as iodine (I), including $^{123}I$, $^{125}I$, $^{131}I$, etc., barium (Ba), gadolinium (Gd), technetium (Tc), including $^{99}Tc$, phosphorus (P), including $^{31}P$, iron (Fe), manganese (Mn), thallium (Tl), chromium (Cr), including $^{51}Cr$, carbon (C), including $^{11}C$, or the like, fluorescently labeled compounds, and fluorine containing chemicals useful in ultrasound, etc.

Taking into account these definitions, the present invention is directed to salicylate molecules modified to include a therapeutic or diagnostic agent.

It is frequently desirable to have the ability to covalently link a particular compound of interest to a specific target, e.g. one of a mixture of components or a site on a macromolecule. For instance, covalent linkage of a therapeutic or diagnostic agent to a specific target macromolecule present in the human vasculature can result in an effective increase in the half-life of that therapeutic or diagnostic agent. Having the ability to specifically and covalently link a compound of interest to a macromolecular target or target

site, can have profound implications for drug design and pharmaceutical therapy in humans.

Surprisingly and in accordance with the present invention, it was found that salicylates can covalently and specifically link the biologically active agent of interest to serum albumin in the presence of other blood components when a sample of whole blood was treated with the salicylates. No labeling of other plasma components (such as IgG) was detected on Western blotting gel (Fig.10. The albumin-specific labeling exhibited by salicylates is an important feature of this invention. The target specificity of salicylates will minimize potential side effects caused by the labeling of other unwanted targets by non-specific compounds.

It was further verified that labeling of albumin occurred specifically at the Lys 199 residue by tryptic digestion and mass spectrometric analysis (Fig.11). Considering that there are about 50 lysine residues on albumin alone, the specificity manifested by these salicylates is remarkable. Furthermore, the drug-albumin conjugates (in this case, ARGATROBAN is the drug) generated from the corresponding salicylates were shown to be biologically active in inhibiting its target enzyme, thrombin (Fig. 9).

In accordance with the present invention, preferred salicylate compounds are provided for extending the life of a biologically active agent of interest, which is a physiologically active agent, in a mammalian host by providing for the specific binding of a biologically active agent of interest to serum albumin.

The salicylate molecule has at least three active moieties:

1) a salicylate moiety for specifically binding the complex to serum albumin;

2) a spacing linker; and

3) a therapeutic or diagnostic agent of interest, which will be specifically bound to the albumin at Lys 199 after the salicylate compound reacts with the albumin.

The "therapeutic agent" may provide for a wide variety of functions. It may serve as an antithrombotic agent, a renin inhibitor, a pain management

agent, an immunogen for the production of antisera or monoclonal antibodies, where one is interested in a diagnostic or therapeutic reagent, for the production of vaccines, or for the protection, prophylactic or therapeutic against a deleterious blood-borne target, such as a pathogen, noxious agent or endogenous factor. The therapeutic agent may react directly with the deleterious blood-borne agent, have antiproliferative activity, particularly cytotoxic activity, by itself or in conjunction with an endogenous system, such as with antibodies to a pathogen or deleterious endogenous cell, e.g. T cell, or to a toxin, may have specific binding activity with a surface membrane receptor, so as to reduce or inhibit the signal transduced by such receptor, may bind to a homing receptor, integrin or addressin, so as to inhibit extravasation or diapedesis, or may change the distribution of the target, including enhancing the excretion of the target by the host or reducing the concentration of the target in one compartment of the body in contrast to a different compartment of the body.

The therapeutic agent is provided at a biologically effective concentration, where the biologically active agent may act in the bound form as part of the reagent.

"Biologically effective concentration" of the biologically active agent means the agent concentration that is bioavailable to the target during the course of the procedure. Biologically effective concentration of the target will mean the immediately bioavailable concentration of the target at the *in vivo* site of its action. For the most part, the target will be at least partially dispersed in the blood stream, may be in solution or associated with serum albumin present in the blood stream. Thus the effective concentration of the target may be reduced by limiting the volume of distribution of the target as to sites at which the target is segregated, by limiting diffusion, mobility, or migration of the target, etc. Thus the physiologically active agent will interact with serum albumin present in the blood stream, so as to provide the desired result. Serum albumin is present in the blood for extended periods of time and is present in a concentration of about 50 mg/ml. The half-life of human albumin is about 18

days. The interaction is a specific interaction which provides for a specific result with a subset of the entities with which the physiological agents interacts.

The disclosed therapeutic methods are applicable to a broad range of targets, both host derived and foreign (meaning exogenous or non-host), which may be present in the blood and have a deleterious physiological effect, due to an undesirably high effective concentration, or as in the case of neoplastic cells, being present in any amount. Host derived cellular targets include, (with parenthetical clinical indication): T cell or subsets, such as αIFN+, CD4+, CD8+, LFA1+, etc., cells (autoimmune disease, alloreactivity, xenoreactivity and inflammation), B cells or subsets such as pre-B cells, CD5+, IgE+, IgM+ etc. (B cell lymphoma, xenograft, autoimmunity, anaphylaxy), leukocytes, such as macrophages and monocytes (inflammation, myelomonocytic leukemia), other leukocytes such as neutrophils, basophils, NK cells, eosinophils, or allo- or xeno-reactive leukocytes, etc. (inflammation, anaphylaxis, transplant rejection), stem cells such as CD34+ cells (polycythemia), fetal red cells, such as Rh+ red cells (prophylactic of anti-Rh immunization after pregnancy in a Rh- mother), malignant cells (malignancies; CALLA) or infected cells, particularly retroviral, e.g. HIV, infected host cells, or the like.

Host derived non-cellular targets include soluble HLA, class I and class II, and non-classical class I HLA (E, F and G) for modulating immunoregulation, soluble T or B cell surface proteins, cytokines, interleukins and growth factors such as IL1, 2, 3, 4, 6, 10, 13, 14 and 15, soluble IL2 receptor, M-CSF, G-CSF, GM-CSF, platelet growth factors, alpha, beta, and gamma- interferons, TNF, NGFs, arachadonic acid metabolites such as prostaglandins, leukotrienes, thromboxane and prostacyclin for cardiovascular diseases, immunoglobulins such as total IgE for anaphylaxy, specific anti-allergen IgE, auto or allo-antibodies for autoimmunity or allo- or xenoimmunity, Ig Fc receptors or Fc receptor binding factors, carbohydrates (gal), natural antibodies involved in allo- or xenorejection, erythropoietin, angiogenesis factors, adhesion molecules, MIF, MAF, complement factors (classical and alternate pathways, including regulatory factors), PAF, ions such as calcium,

potassium, magnesium, aluminum, iron, etc, enzymes such as proteases, kinases, phosphatases, DNAses, RNAses, lipases and other enzymes affecting cholesterol and other lipid metabolism, esterases, dehydrogenases, oxidases, hydrolases, sulphatases, cyclases, transferases, transaminases, atriopeptidases, carboxylases and decarboxylases and their natural substrates or analogs, superoxide dismutase, hormones such as TSH, FSH, LH, thyroxine (T4 and T3), renin, insulin, apolipoproteins, LDL, VLDL, cortisol, aldosterone, estriol, estradiol, progesterone, testosterone, dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S), calcitonin, parathyroid hormone (PTH), human growth hormone (hGH), vasopressin and antidiuretic hormone (ADH), prolactin, ACTH, LHRH, THRH, VIP, cathecolamines (adrenaline, vanillylmandelic acid, etc.), bradykinins and corresponding prohormones, metabolites, ligands or natural cell or soluble receptors thereof, cofactors including atrionatriuretic factor (ANF), vitamins A, B, C, D, E and K, serotonin, coagulation factors, e.g. prothrombin, thrombin, fibrin, fibrinogen, Factor VIII, Factor XI, Willebrand factor, plasminogen factors, e.g. plasmin, complement activation factors, LDL and ligands thereof, uric acid, etc. In some instances one may provide specific effects associated with complement, by having inhibitors such as DAF, CD59, etc., compounds regulating coagulation, such as hirudin, hirulog, hementin, TPA, etc. or other compounds, such as tissue factor, nucleic acids for gene therapy, etc., compounds which are enzyme antagonists, compounds binding ligands, such as cytokines, hormones, inflammation factors (PAF, complementation factors), etc.

Foreign targets include toxins such as heavy metals like mercury and lead, free radicals, arsenic, bacterial toxins such as LPS and other gram negative toxins, *Staphylococcus* toxins, Toxin A, *Tetanus* toxins, *Diphtheria* toxin and *Pertussis* toxins, plant and marine toxins, snake and other venom, virulence factors, such as aerobactins, radioactive compounds or pathogenic microbes or fragments thereof, including infectious viruses, such as hepatitis , A, B, C, E and delta, CMV, HSV (types 1, 2 & 6), EBV, varisella zoster virus (VZV), HIV-1, -2 and other retroviruses, adenovirus, rotavirus, influenzae, rhinovirus, parvovirus, rubella, measles, polio, reovirus, orthomixovirus,

paramyxovirus, papovavirus, poxvirus and picornavirus, prions, plasmodia tissue factor, protists such as toxoplasma, filaria, kala-azar, bilharziose, entamoeba histolitica and giardia, and bacteria, particularly gram-negative bacteria responsible for sepsis and nosocomial infections such as *E. coli*, *Acynetobacter*, *Pseudomonas*, *Proteus* and *Klebsiella*, but also gram positive bacteria such as *Staphylococcus*, *Streptococcus*, etc. *Meningococcus* and *Mycobacteria*, *Chlamydiae*, *Legionnella* and *Anaerobes*, fungi such as *Candida*, *Pneumocystis carinii*, and *Aspergillus*, and Mycoplasma such as *Hominis* and *Ureaplasma urealyticum*.

Human serum albumin has a half-life of at least about 18 days. Generally, half-life of albumin is determined by serial measurements of whole blood, plasma or serum levels of the compound following labeling of the compound with an isotope (e.g. $^{123}I$, $^{125}I$, $^{131}I$, barium (Ba), gadolinium (Gd), technetium (Tc), including $^{99}Tc$, phosphorus (P), including $^{31}P$, iron (Fe), manganese (Mn), thallium (Ti), chromium (Cr), including $^{51}Cr$, carbon (C), including $^{11}C$, $^{3}H$, or the like, fluorescently labeled compounds, etc.) or fluorochrome and injection of a known quantity of labeled compound i.v. In addition to preferred half-lives, the serum albumin is present in a concentration sufficient to allow binding of therapeutically useful amounts of the conjugate, such as a serum albumin concentration of at least 100μg/ml, usually at least about 0.01 mg/ml, more usually at least about 40 mg/ml (total albumin), are preferred.

Illustrative salicylates compounds of the present invention include renin inhibitor salicylates, calcium channel blocker salicylates, biotin salicylates and argatroban salicylates, such as those shown in Figs. 1-8.

The salicylate compound of the present invention will usually be administered as a bolus, but may be introduced slowly over time by infusion using metered flow, or the like. Alternatively, although less preferable, blood may be removed from the host, treated *ex vivo*, and supplied to the host. The compound will be administered in a physiologically acceptable medium, e.g. deionized water, phosphate buffered saline, saline, mannitol, aqueous glucose, alcohol, vegetable oil, or the like. Usually a single injection will be employed

although more than one injection may be used, if desired. The compound may be administered by any convenient means, including syringe, trocar, catheter, or the like. The particular manner of administration, will vary depending upon the amount to be administered, whether a single bolus or continuous administration, or the like. For the most part the administration will be intravascularly, where the site of introduction is not critical to this invention, preferably at a site where there is rapid blood flow, e.g. intravenously, peripheral or central vein. Other routes may find use where the administration is coupled with slow release techniques or a protective matrix. The intent is that the compound be effectively distributed in the blood, so as to be able to react with the blood components.

For the most part, reaction will be with mobile serum albumin in the blood. By "mobile" is intended that the component does not have a fixed situs for any extended period of time, generally not exceeding 5, more usually one minute.

The dosage of the compound will depend upon its activity when bound to blood components, the time necessary to reduce the free concentration of the compound, the dosage necessary for therapeutic activity, the indication being treated, the sensitivity of the agent to blood enzymes, the route and mode of administration, and the like. As necessary, the dosage may be determined empirically, initially using a small multiple of the therapeutic dosage normally administered, and as greater experience is obtained, enhancing the dosage.

The salicylate compounds of the present invention may be synthesized according to the synthesis schemes illustrated in the following examples.

The salicylate compounds of the present invention may be prepared in any convenient way, depending on the nature of the biologically active agents, the spacing linker group of the compound, the need to maintain the bioactivity of biologically active agents and such other considerations as are relevant to the use of the compound *ex vivo* and *in vivo*.

The salicylate compounds of the present invention may be used for the treatment, prophylactic or therapeutic, of a wide variety of cellular diseases, toxicities and environmental exposures and may be administered in a wide variety of ways, depending on the indication. If desired, the salicylate compounds may be first bound to albumin in a 1:1 ratio followed by administration. Alternatively, the salicylate compounds may be administered to the host for binding to serum albumin. The amount of the conjugate which is administered will vary widely, depending upon the nature of the compound, the purpose for the compound, the therapeutic dosage, the physiological activity of the compound, and when bound to a cell, and the like. Therefore, the amount of conjugate administered to a host may vary from 1 pg to 50 mg/kg of host.

The salicylate compounds of the present invention will, for the most part, be administered parenterally, such as intravascularly (IV), intraarterially, intramuscularly (IM), subcutaneously (SC), or the like. Administration will normally be by transfusion if the compound is bound to albumin. If the compound is unbound, administration will normally be IV, IM or SC. Where the compositions are of low molecular weight (less than about 10 kD) or resistant to digestive enzymes, compound administration may be oral, nasal, rectal, transdermal or aerosol, where the nature of the compound allows for transfer to the vascular system. Physiologically acceptable carriers will usually be employed, such as water, saline, phosphate buffered saline, aqueous ethanol, plasma, proteinaceous solutions, glucose or mannitol solutions, or the like. The concentration of the conjugate will vary widely, generally ranging from about 1 pg/ml to 50 mg/ml. Other additives which may be included include buffers, where the media are generally buffered at a pH in the range of about 5 to 10, where the buffer will generally range in concentration from about 50 to 250 mM, salt, where the concentration of salt will generally range from about 5 to 500 mM, physiologically acceptable stabilizers, and the like. The compositions may be lyophilized for convenient storage and transport.

The subject invention may be used in chronic or acute situations, either prophylactic or therapeutic.

Because of the extended delivery time or availability of the subject agents, the subject invention may be used in a wide variety of situations. The subject invention may be employed before surgery so as to ensure that a level of drug is maintained during and subsequent to the surgery without requiring repetitive administration, avoiding the disturbance of the patient. For example, one may use anticlotting agents, where the nature of the surgery and indication is susceptible to the formation of clots. One may use inhibitors of leukocyte homing to prevent perfusion injury. One may use the subject invention with cardiovascular drugs, where a patient is particularly susceptible during an extended period to myocardial infarction. Other treatments which will benefit from long term availability of drugs include hormonotherapy, infertility therapy, immunosuppressive therapy, neuroleptic therapy, drug of abuse prophylaxy, treatment of diseases caused by infectious agents, treatment of hemophilia, and the like.

The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

## EXAMPLE I

### Synthesis of Arg-C6 5-Bromo Salicylic Acid (YS-41-13)

The synthesis scheme 1 is illustrated in Fig. 2.

Methyl 6-Aminohexanoate Hydrochloride (YS-004-58)

To a suspension of 6-aminocaproic acid (3.00 g, 22.9 mmol) in anhydrous MeOH (60 ml), was introduced hydrogen chloride gas for 25 min., during which time the suspension became clear. The solution was then stirred at room temperature for 5 hours. MeOH was removed *in vacuo*. The residue was recrystallized from THF to give a white solid (3.10 g). (Yield: 93%).

$^1$HNMR (300 MHz, $CD_3OD$) $\delta$ 3.66 (s, 3H, $OCH_3$), 2.92 (t, 2H, J = 7.7 Hz, $NCH_2$), 2.37 (t, 2H, J = 7.2 Hz, $CH_2CO_2$), 1.74 - 1.60 (m, 4H, $CH_2CH_2$), 1.48 - 1.36 (m, 2H, $CH_2$).

Argatroban-C6 Methyl Ester (YL-015-13)

To a solution of argatroban monohydrate (100 mg, 0.190 mmol) and methyl 6-aminohexanoate hydrochloride (YS-004-58) (35 mg, 0.193 mmol) in anhydrous DMF (5 ml), was added triethylamine (23 mg, 0.23 mmol), followed by addition of HBTU (86 mg, 0.23 mmol). Stirring was continued at room temperature for 20 hours. The precipitates formed during the reaction were filtered off. DMF was removed by vacuum distillation. The residue was purified by preparative TLC using solvents $CH_2Cl_2$/MeOH/$NH_3$ (90/10/1, v/v) to give the titled compound (78 mg) (Yield: 65%). $^1$HNMR (300 MHz, $CD_3OD$) (rotamer ratio: 55 : 45) d 7.48 and 7.45 (rotamers, d, 1H, J = 8 Hz), 7.13 and 7.10 (rotamers, d, 1H, J = 8 Hz), 6.61 - 6.51 (m, 1H), 5.19 - 5.12 and 4.47 - 4.39 (rotamers, m, 1H), 4.27 - 4.07 (m, 1H), 3.99 - 3.80 (m, 1H), 3.648 and 3.639 (rotamers, s, 3H, $OCH_3$), 3.51 - 3.33 (m, 1H), 3.30 - 2.65 (m, 7H), 2.52 - 2.38 (m, 1H), 2.32 and 2.31 (rotamers, t, 2H, J = 8 Hz, $CH_2CO_2CH_3$), 2.09 - 1.90 (m, 1.5H), 1.78 - 1.23 (m, 12.5H), 1.18 - 0.97 (m, 1.5H), 1.07 (d, 3H, J = 7 Hz, $CH_3$), 0.93 and 0.89 (rotamers, d, 3H, J = 7 Hz, $CH_3$), 0.76 - 0.55 (m, 0.5H). MS (electrospray) 636.4 ($M+H^+$) (635.3 calcd. for $C_{30}H_{49}N_7O_6S$).

Argatroban-C6 Free Acid (YL-015-15)

A solution of compound YL-015-13 (124 mg, 0.159 mmol) in MeOH (3 ml) and 1 M aqueous NaOH (0.55 ml) was stirred at room temperature for 33 hours. The reaction was worked up by the same procedures as described for preparation of compound YL-015-10, to give the titled compound (76 mg) (Yield: 73%). $^1$HNMR (300 MHz, $CD_3OD$) (rotamer ratio: 55 : 45) d 7.48 and 7.45 (rotamers, d, 1H, J = 8 Hz), 7.13 and 7.10 (rotamers, d, 1H, J = 8 Hz), 6.61 - 6.50 (m, 1H), 5.19 - 5.11 and 4.47 - 4.37 (rotamers, m, 1H), 4.27 - 4.07 (m, 1H), 3.99 - 3.79 (m, 1H), 3.52 - 2.65 (m, 8H), 2.54 - 2.35 (m, 1H), 2.314 and 2.300 (rotamers, t, 2H, J = 7 Hz, $CH_2CO_2$), 2.10 - 1.88 (m, 1.5 H), 1.80 - 1.28 (m, 12.5 H), 1.18 - 0.94 (m, 1.5 H), 1.07 (d, 3H, J = 7 Hz, CH3), 0.94 and 0.88 (rotamers, d, 3H, J = 7 Hz, CH3), 0.76 - 0.55 (m, 0.5 H). MS (electrospray) 622.1 ($M+H^+$) (621.3 calcd. for $C_{29}H_{47}N_7O_6S$).

Argatroban-C6 NHS Ester (YL-015-35)

To a solution of compound YL-015-15 (78 mg, 0.12 mmol) and N-hydroxysuccinamide (29 mg, 0.25 mmol) in anhydrous DMF (6 ml), was added EDC (72 mg, 0.38 mmol). The solution was stirred at room temperature for 20 hours. DMF was removed by vacuum distillation. The residue was dissolved in a minimum amount of MeOH (0.4 ml). $H_2O$ (1.2 ml) was added to induce precipitation. The precipitates were washed with $H_2O$ (3 x 0.7 ml), and dried on vacuum to afford a solid. It was further purified by recrystallization from acetone/ether (1/1, v/v) to give a white solid product (62 mg) (Yield: 69%). $^1$HNMR (300 MHz, $CD_3OD$) (rotamer ratio: 55 : 45) d 7.48 and 7.45 (rotamers, d, 1H, J = 8 Hz), 7.13 and 7.10 (rotamers, d, 1H, J = 8 Hz), 6.61 - 6.50 (m, 1H), 5.19 - 5.11 and 4.52 - 4.42 (rotamers, m, 1H), 4.27 - 4.07 (m, 1H), 4.00 - 3.80 (m, 1H), 3.52 - 2.65 (m, 8H), 2.87 and 2.82 (rotamers, s, 4H, $OCCH_2CH_2CO$), 2.630 and 2.617 (rotamers, t, 2H, J = 8 Hz, $CH_2CO_2N$), 2.54 - 2.35 (m, 1H), 2.10 - 1.88 (m, 1.5 H), 1.80 - 1.28 (m, 12.5 H), 1.18 - 0.94 (m, 1.5 H), 1.07 (d, 3H, J = 7 Hz, $CH_3$), 0.94 and 0.88 (rotamers, d, 3H, J = 7 Hz, $CH_3$), 0.76 - 0.55 (m, 0.5 H). MS (electrospray) 719.4 ($M+H^+$) (718.3 calcd. for $C_{33}H_{50}N_8O_8S$).

Argatroban-C6 5-Bromo Salicylic t-Butyl Ester (YS-41-12A)

A solution of 5-bromo salicylic t-butyl ester (197 mg, 0.72 mmol) and potassium t-butoxide (81 mg, 0.72 mmol) in anhydrous THF (1.0 ml) was quickly mixed with the powder of Argatroban-C6 NHS ester (54 mg, 72 mmol). The solution was then stirred at 22°C for 20 min.. The solvent was removed *in vacuo*. $H_2O$ was added, and the solution was acidified with acetic acid to pH 4-5. The precipitates were collected by vacuum filtration, then dissolved in MeOH. The solution was washed with hexane three times to remove excess of 5-bromo salicylic t-butyl ester, and then concentrated *in vacuo* to give a solid (35 mg), which was purified by HPLC using a gradient of $CH_3CN$ in $H_2O$ as eluents varying from 10% to 100% over 55 min.. The positive fractions were pooled and lyophilized to give a powder (2.5 mg) (Yield: 4%). MS

(electrospray) 876.4 and 878.4 ($M+H^+$, bromine isotope pattern) (ave. 876.9 calcd. for $C_{40}H_{58}N_7O_8SBr$).

Argatroban-C6 5-Bromo Salicylic Acid (YS-41-13)

Compound YS-41-12A (1.2 mg) was dissolved in TFA (1ml) at room temperature. The solution was allowed to stand at room temperature for 2 hours. TFA was removed *in vacuo* to give a solid (1.0 mg). MS (electrospray) 820.4 and 822.4 ($M+H^+$, bromine isotope pattern) (ave. 820.8 calcd. for $C_{36}H_{50}N_7O_8SBr$).

## EXAMPLE II

### Synthesis of Arg-C12 5-Bromo Salicylic Acid (QC-37-49)

The synthesis of this compound was performed according to scheme 1 (Fig. 2) but using methyl 6-aminododecanoate hydrochloride instead of methyl 6-aminohexanoate hydrochloride. All the other steps of the synthesis are similar to the preparation of YS-41-13. The final compound QC-37-49 was obtained with a global yield of 5% based on argatroban, similar to the global yield obtained for the synthesis of YS-41-13.

## EXAMPLE III

### Synthesis of Arg-C18 PE Salicylic Acid (YS-41-94)

The synthesis scheme 2 is illustrated in Figs. 3A and 3B.

Compound YS-41-53

To a solution of 2-[2-(2-chloroethoxy)ethoxy] ethanol (1.69 g, 10 mmol) and t-butyl bromoacetate (1.95 g, 10 mmol) in anhydrous THF (22 ml), potassium t-butoxide (1.18 g, 95%, 10 mmol). The mixture was stirred at room temperature overnight. Water and ethyl acetate were added. Organic phase was separated, and aqueous phase was extracted with ethyl acetate. The combined organic solutions were dried over $Na_2SO_4$, then concentrated *in vacuo* to give a brownish liquid, which was purified by flash column using

solvents EtOAc/hexanes (30/70) to afford a clear liquid (0.95 g) (YS-41-53) (yield: 33%).

Compound YS-41-54

To a solution of the chloride YS-41-53 (0.92 g, 3.26 mmol) in anhydrous DMF (30 ml), sodium azide (1.04 g, 16 mmol) was added. The suspension was then heated to 80°C overnight. Ethyl acetate was added and the solution was washed with $H_2O$. The organic phase was dried over $Na_2SO_4$, concentrated *in vacuo* to give a yellowish liquid (0.90 g) (YS-41-54) (yield: 96%).

Compound YS-41-71

The t-butyl ester YS-41-54 (0.438 g, 1.52 mmol) was dissolved in TFA (6 ml). The solution was allowed to stand at room temperature for 1 hour. TFA was removed *in vacuo*. The residue was purified by a silica gel column using $CH_2Cl_2$/MeOH/$CH_3CO_2H$ (90/10/1) as eluents. The fractions containing the compound were pooled, concentrated *in vacuo*, dried on vacuum overnight to remove residual $CH_3CO_2H$ that would interfere with the next reaction. An oil was obtained (0.186 g) (yield: 53%).

Compound YS-41-73

To a solution of the acid YS-41-71 (0.180 g, 0.773 mmol) and HOBt (0.125 g, 0.926 mmol) in $CH_2Cl_2$ (7 ml), DCC (0.177 g, 0.859 mmol) was added. The mixture was stirred at room temperature for 1 hour. A solution of the amino ester hydrochloride YS-004-58 (0.140 g, 0.771 mmol) and triethylamine (130 ml, 0.933 mmol) in $CH_2Cl_2$ (4 ml) was added dropwise. The mixture was stirred at room temperature overnight. The solution was filtered, and the filtrate was washed with 5% $NaHCO_3$, then $H_2O$. It was dried over $Na_2SO_4$, concentrated *in vacuo* to give an oil (0.248 g) (yield: 89%).

Compound YS-41-74

To a solution of Triphenylphosphine(0.196 g, 0.747 mmol) and $H_2O$ (40 ml, 2.22 mmol) in THF (3 ml), the azide YS-41-73 (0.240 g, 0.667 mmol) was added. The solution was stirred at room temperature overnight. The solvent was removed *in vacuo*. The residue was purified by a silica gel column, which was eluted first with $CH_2Cl_2$, then with 20% MeOH in $CH_2Cl_2$. The positive fractions were pooled, concentrated *in vacuo* to give an oil (0.100 g) (yield: 45%). MS (electrospray) 335.6 ($M+H^+$) (334.2 calcd. for $C_{15}H_{30}N_2O_6$).

Compound YS-41-75

To a solution of argatroban (0.157 g, 0.298 mmol) and the amine YS-41-74 (0.100 g, 0.299 mmol) in anhydrous DMF (6 ml), HBTU (0.136 g, 0.359 mmol) was added. The mixture was stirred at room temperature overnight. DMF was removed *in vacuo*. The residue was purified by HPLC using a gradient of $CH_3CN$ in $H_2O$ (containing 0.045% TFA) as eluents varying from 10% to 100% over 55 min. The positive fractions were pooled and lyophilized to give a white powder (0.144 g) (yield: 52%).

Compound YS-41-86

A solution of the methyl ester YS-41-75 (66 mg, 0.070 mmol) in MeOH (2 ml) and 1M NaOH (0.4 ml, 0.4 mmol) was stirred at room temperature overnight. MeOH was removed *in vacuo*. The aqueous solution was neutralized by 1M HCl (0.4 ml) to pH3-4. The formed precipitates were collected by filtration. The filtrate was lyophilized, and the residue was washed with MeOH to extract the product. The combined product sample was purified by HPLC using a gradient of $CH_3CN$ in $H_2O$ (containing 0.045% TFA) as eluents varying from 10% to 100% over 55 min. The positive fractions were pooled and lyophilized to give a white powder (51 mg) (yield: 78%).

Compound YS-41-89

To a solution of the acid YS-41-86 (51 mg, 0.055 mmol), methylthiomethyl 5-bromosalicylate QC-37-43B (30 mg, 0.108 mmol) and DIEA (56 ml, 0.322 mmol) in anhydrous DMF (2 ml), HBTU (45 mg, 0.119 mmol) was added. The solution was stirred at room temperature for 3 days. DMF was removed *in vacuo*. The residue was washed with $Et_2O$ to remove excess of methylthiomethyl 5-bromosalicylate. It was then purified by HPLC using a gradient of $CH_3CN$ in $H_2O$ (containing 0.045% TFA) as eluents varying from 10% to 100% over 55 min. The positive fractions were pooled and lyophilized to give a white powder (10 mg) (yield: 16%).

Compound QC-37-43B was prepared as followed: To a solution of 5-bromosalicylic acid (1.00g, 4.60 mmol) in THF (20 ml), potassium t-butoxide (0.516 g, 4.60 mmol) was added. The solution was stirred at room temperature for 10 min. It was then concentrated *in vacuo*. The residue was dissolved in toluene (20 ml) and DMF (5 ml). To the solution, methylthiomethyl chloride (0.385 ml, 4.60 mmol), sodium iodide (0.680 g, 4.60 mmol) and 18-crown-6 (0.120 g, 0.460 mmol) were added. The suspension was heated to reflux for 3 hour. It was then cooled down, and stirred at room temperature for 3 days. The solvents were removed *in vacuo*. The residue was dissolved in EtOAc. The solution was washed with $H_2O$ twice, dried over $MgSO_4$, concentrated *in vacuo* to give a brown residue, which was purified by a silica gel column using hexane / EtOAc (98 / 2) as eluents to afford a solid (0.650g) (yield: 51%).

Compound YS-41-94

The ester YS-41-89 (7 mg, 5.9 mmol) was dissolved in TFA (2 ml) containing phenol (12 mg, 0.128 mmol). The solution was allowed to stand at room temperature for 30 min. TFA was removed *in vacuo*. The residue was washed with $Et_2O$ twice to remove excess of phenol. It was then purified by HPLC using a gradient of $CH_3CN$ in $H_2O$ (containing 0.045% TFA) as eluents varying from 10% to 100% over 55 min. The positive fractions were pooled

and lyophilized to give a white powder (2.4 mg) (yield: 36%). MS (electrospray) 1011.4 ($M+H^+$) (1010.0 calcd. for $C_{44}H_{65}N_8O_{12}SBr$).

## EXAMPLE IV

**Synthesis of Biotin 5-Bromosalicylic Acid (QC-28-31)**

The synthesis scheme is illustrated in Fig. 4.

Compound YS-004-71

To a solution of biotin (2.00 g, 8.19 mmol) and 2-nitrophenylsulfonate (2.42 g, 9.01 mmol) in DMF (20 ml), DCC (1.86 g, 9.01 mmol) was added. The reaction mixture was heated to 60°C and stirred at that temperature for 2 hour. It was then stirred at room temperature overnight. The solution was filtered, and the filtrate was concentrated *in vacuo*. The residue was dissolved in MeOH, $Et_2O$ was added to induce precipitation. The precipitate was collected by vacuum filtration. It was dissolved in $H_2O$, and the solution was kept in refrigerator to precipitate out all DCU. It was then filtered, and the filtrate was concentrated *in vacuo*. The residue was dissolved in MeOH, and $H_2O$ was added to induce precipitation. The yellow precipitate was collected (2.00 g) (yield: 52%)

Compound QC-28-30

To a solution of 5-bromosalicylic acid (1.00 g, 4.60 mmol) and DMAP (22.4 mg, 0.184 mmol) in t-butyl alcohol (25 ml), DCC (1.05 g, 5.07 mmol) in THF (5 ml) was added. The mixture was stirred at room temperature overnight. The solution was filtered, and the filtrate was concentrated *in vacuo*. The product was taken up in hexane. The solution was dried over $Na_2SO_4$, concentrated *in vacuo*. The residue was recrystallized form MeOH to afford white crystals (0.400 g) (yield: 32%).

Compound QC-28-31

To a solution of biotin nitrophenylsulfonate YS-18- (0.100 g, 0.215 mmol) and DIEA (37 ml, 0.215 mmol) in DMF (5 ml), a mixture of the ester QC-28-30 (0.117 g, 0.430 mmol) and potassium t-butoxide (48.3 mg, 0.430

mmol) in anhydrous THF was added. The reaction mixture was stirred at room temperature for 0.5 hour. The solvents were removed *in vacuo*. Water and $CHCl_3$ were added to dissolve the residue. The $CHCl_3$ phase was separated, washed with 10% $KHSO_4$, $H_2O$, 5% $NaHCO_3$, $H_2O$, then dried over $MgSO_4$, concentrated *in vacuo* to give a solid (51 mg). The compound was then dissolved in TFA (2 ml). The solution was allowed to stand at room temperature for 1 hour. TFA was removed *in vacuo* to afford a solid (43 mg) (yield: 52%).

The Western blot illustrated in Fig. 9, indicate that biotin-salicylate specifically labels human serum albumin in whole blood.

## EXAMPLE V

### Synthesis of Boc-Phe-His-ACHPA-Ile-Ac-Salicylate

The synthesis scheme is illustrated in Figs. 5A and 5B.

Abbreviations used are as follows: DCC, dicyclohexylcarbodiimide; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; NHS, N-hydroxysuccinimide; sulfo-NHS, 3-sulfo-N-hydroxysuccinimide; DMF, dimethylformamide; Boc, tert-butyloxycarbonyl; TFA, trifluoroacetic acid; DNP, dinitrophenyl; AC, aminocaproic; ACHPA, (3S,4S)-4-amino-3-hydroxy-5-cyclohexylpentanoic acid; P-EDC, polymer bound 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide.

All chemicals and solvents are reagent grade or higher, which are purchased from Aldrich Chemical Co. (Milwaukee, WI). Boc-Phe-DNP-His is purchased from Sigma Chemical Co. (St. Louis, MO). Boc-ACHPA is synthesized according to literature procedure (Ref: Boger, J., Payne, L. S., Perlow, D. S., Lohr, N. S., Poe, M., Blaine, E. H., Ulm, E. H., Schorn, T. W., LaMont, B. I., Lin, T-Y., Kawai, M., Rich, D. H., Veber, D. F., *J. Med. Chem.*, **1985**, *28*, 1779-1790). P-EDC is prepared according to literature report (Ref. Desai, M. C. and Stephens Stramiello, L. M., *Tetra. Lett.*, **1993**, *34*, 7685-7688).

NMR spectra are recorded on a Varian Gemini 2000 spectrometer (300 MHz), and are expressed in ppm from tetramethylsilane. Mass

spectrometry are performed on a Perkin-Elmer API 300. HPLC analyses and preparation are performed on a Hewlett-Packard 1050 and a Rainin instrument, respectively. Thin layer chromatography (TLC) is done on silica gel plates (E. Merck) and components are visualized by ninhydrin spray after the plates were kept at HCl gas chamber for 15 min to cleave Boc group. Column chromatography is performed on silica gel 60, 0.04-0.063 mm (E. Merck).

Methyl 6-Aminocaproic (AC) Ester Hydrochloride (2, 6-AC-OMe).

To a suspension of 6-aminocaproic acid (1, 3.00 g, 22.9 mmol) in anhydrous methanol (60 mL), hydrogen chloride gas is introduced for 25 min., during which time the suspension will become clear. The solution is then stirred at room temperature for 5 hours. Methanol is removed in vacuo. The residue is recrystallized from THF to give a white solid (2, 3.10 g). (Yield: 75%). Compound 2 is better stored in hydrochloride form, because its free amino form tends to polymerize. Estimate NMR spectra are $^1$HNMR ($CD_3OD$, 300 MHz) □ 3.66 (s, 3H, $OCH_3$), 2.92 (t, 2H, J = 7.7 Hz, $NCH_2$), 2.37 (t, 2H, J = 7.2 Hz, $CH_2CO_2$), 1.74 - 1.60 (m, 4H, $CH_2CH_2$), 1.48 - 1.36 (m, 2H, $CH_2$).

Boc-Ile-6-AC-OMe (3) and $H_2$N-Ile-6-AC-OMe (4).

To a solution of N-Boc isoleucine (2.55 g, 11.0 mmol) and HOBT (1.49 g, 11.0 mmol) in $CH_2Cl_2$ (250 mL) at 22°C, DCC (2.38 g, 11.6 mmol) is added. The mixture is stirred at 22°C for 3 hour. A solution of methyl 6-aminocaproate hydrochloride (2, 2.00 g, 11.0 mmol) and triethylamine (1.55 mL, 11.1 mmol) in anhydrous THF (25 mL) is added. The reaction mixture is then stirred at 22°C overnight. The solvent is removed in vacuo. Ethyl ether is added, and the insoluble is filtered off. The etheral solution is washed with 5% $NaHCO_3$, $H_2O$. It is then dried over $Na_2SO_4$, concentrated in vacuo to give an amorphous solid ( 3, 3.05 g) (yield: 77%). NMR spectra would be $^1$HNMR (300 MHz, $CDCl_3$) • 6.00 (br. s, 1H, NH), 5.03 (br. s, 1H, NH), 3.86 (dd, 1H, J = 8, 6 Hz, NCHCO), 3.68 (s, 3H, $OCH_3$), 3.32 - 3.20 (m, 2H, $NCH_2CH_2$), 2.32 (t, 2H, J = 8 Hz, $CH_2CO_2$), 1.98 - 1.03 (m, 9H, $CH_2$), 1.45 (s,

9H, $(CH_3)_3C$), 0.96 - 0.84 (m, 6H, $2CH_3$). MS (electrospray) 359.5 ($M+H^+$), 717.7 ($2M+H^+$) (358.2 calcd. for $C_{18}H_{34}N_2O_5$).

Boc-Ile-6-AC-OMe (3, 3.00 g, 8.38 mmol) is dissolved in trifluoroacetic acid (30 mL). The solution is stirred at 22°C for 2 hours. Trifluoroacetic acid is removed in vacuo. The residue is dissolved in a minimum amount of THF, $H_2O$ is added. The solution is then lyophilized to give an oil ($H_2N$-Ile-6-AC-OMe) (4, 3.11 g trifluoroacetate salt) (representative yield: 99%).

Boc-ACHPA-Ile-6-AC-OMe (6) and $H_2N$-ACHPA-Ile-6-AC-OMe (7).

To a solution of Boc-ACHPA (5 , 2.64 g, 8.38 mmol) and HOBT (1.24 g, 9.19 mmol) in $CH_2Cl_2$ (80 mL) at 22°C, DCC (1.89 g, 9.17 mmol) is added. The solution is stirred at 22°C for 4 hours. A solution of $H_2N$-Ile-6-AC-OMe (4, 3.11 g, 8.37 mmol) and triethylamine (2.32 mL, 16.7 mmol) in $CH_2Cl_2$ (30 mL) is added. The reaction mixture is stirred at 22°C overnight. The precipitates are filtered off, and the filtrate is washed with 5% $NaHCO_3$ twice, then with $H_2O$. The solution is dried over $Na_2SO_4$, concentrated in vacuo to give a solid residue, which is purified by a flash silica gel column using solvents ethyl acetate / hexane (70 / 30) to give a white amorphous solid (6, 2.66 g) (yield: 57%). NMR spectra are $^1$HNMR (300 MHz, $CDCl_3$) • 6.48 (d, 1H, J = 10 Hz, NH), 6.39 (br.t, 1H, J = 5 Hz, N*H*$CH_2$), 4.76 (d, 1H, J = 10 Hz, NH), 4.26 (dd, 1H, J = 10, 8 Hz, •-H of Ile), 4.04 - 3.93 (br.m, 1H, C*H*OH), 3.75 (d, 1H, J = 3 Hz, OH), 3.66 (s, 3H, $OCH_3$), 3.65 - 3.53 (br.m, 1H, NC*H*CHOH), 3.25 (dt, 2H, J = 5, 6 Hz, N$CH_2CH_2$), 2.55 - 2.41 and 2.39 - 2.23 (m, 2H, HOCHC$H_2$CO), 2.30 (t, 2H, J = 8 Hz, $CH_2CO_2$), 1.98 - 1.03 (m, 19H), 1.46 (s, 9H, $(CH_3)_3C$), 1.02 - 0.73 (m, 9H). MS (electrospray) 556.6 ($M+H^+$), 1112.1 ($2M+H^+$) (555.4 calcd. for $C_{29}H_{53}N_3O_7$).

Boc-ACHPA-Ile-6-AC-OMe (6, 2.64 g, 4.76 mmol) is dissolved in trifluoroacetic acid (35 mL). The solution is stirred at 22°C for 2 hours. Trifluoroacetic acid is removed in vacuo. The residue is dissolved in ethyl acetate (150 mL). The solution is washed with 5% $NaHCO_3$ three times, then with $H_2O$. It is dried over $Na_2SO_4$, concentrated in vacuo to give a solid ($H_2N$-ACHPA-Ile-6-AC-OMe) (7, 2.05 g) (yield: 95%).

Boc-Phe-DNP-His (8).

Boc-DNP-His (contains 1 mol of isopropanol, 6.69 g, 13.9 mmol) is dissolved in trifluoroacetic acid (40 mL). The solution is stirred at 22°C for 1 hour. Trifluoroacetic acid is removed in vacuo. The residue is dissolved in $H_2O$ (40 mL), and it is lyophilized to give a greenish solid ($H_2N$-DNP-His).

To a solution of Boc-Phe (3.68 g, 13.9 mmol) and HOBT (1.88 g, 13.9 mmol) in $CH_2Cl_2$ (100 mL) at 22°C, DCC (2.88 g, 14.0 mmol) in $CH_2Cl_2$ (30 mL) is added. The reaction mixture is stirred at 22°C for 5 hours. The precipitates are filtered off, and the filtrate is concentrated in vacuo. To the residue, a solution of $H_2N$-DNP-His prepared above in anhydrous DMF (80 mL) is added, followed by addition of triethylamine (5.80 mL, 41.7 mmol). The reaction mixture is then stirred at 22°C overnight. Water and ethyl acetate are added. The organic layer is separated, and the aqueous layer is extracted with ethyl acetate once more. The combined organic phases are extracted with 5% $NaHCO_3$. To the $NaHCO_3$ solution, 6N HCl is added to adjust the pH to 2 to 3. The product is then extracted with ethyl acetate. The ethyl acetate solution is dried over $Na_2SO_4$, concentrated in vacuo to give a yellowish powder (8, 5.18 g) (yield: 66%). NMR spectra are: $^1$HNMR (300 MHz, $CDCl_3$) • 8.92 (d, 1H, J = 3.5 Hz, ArH of DNP), 8.59 (dd, 1H, J = 3.5, 8 Hz, ArH of DNP), 7.84 (br.s, 1H, ArH of imidazole), 7.80 (d, 1H, J = 8 Hz, ArH of DNP), 7.30 - 7.10 (m, 5H, PhH), 6.90 (br.s, 1H, ArH of imidazole), 6.73 (br.s, 1H, NH), 5.10 (d, 1H, J = 8 Hz, NH), 4.70 (br.s, 1H, •-H of His), 4.36 (dt, 1H, J = 7, 8 Hz, •-H of Phe), 3.44 - 3.23 (m, 2H, $CH_2$-imidazole), 3.07 (d, 1H, J = 7 Hz, $CH_2Ph$), 1.32 (s, 9H, $(CH_3)_3C$). MS (electrospray) 569.4 ($M+H^+$), 1137.6 ($2M+H^+$) (568.2 calcd. for $C_{26}H_{28}N_6O_9$).

Boc-Phe-DNP-His-ACHPA-Ile-6-AC-OMe (9).

To a solution of Boc-Phe-DNP-His (8, 2.55 g, 4.49 mmol) and HOBT (0.606 g, 4.49 mmol) in $CH_2Cl_2$ (60 mL), DCC (0.943 g, 4.58 mmol) is added. The solution is stirred at 22°C for 1 hour. A solution of $H_2N$-ACHPA-Ile-6-AC-OMe (2.04 g, 4.48 mmol) in $CH_2Cl_2$ (15 mL) is added. The mixture is then

stirred at 22°C overnight. The precipitates are filtered off. The filtrate is washed with 5% $NaHCO_3$, then $H_2O$. It is dried over $Na_2SO_4$, concentrated in vacuo to give a yellow solid (3.97 g), which could be used without further purification. For a pure sample, it (108 mg) could be purified on a flash silica gel column using solvents $CH_2Cl_2$ / MeOH (95 / 5) to afford a yellowish solid (9, 78 mg). NMR spectra are: $^1$HNMR (300 MHz, $CDCl_3$) • 8.87 (d, 1H, J = 3.5 Hz, ArH of DNP), 8.59 (dd, 1H, J = 3.5, 8 Hz, ArH of DNP), 8.38 (d, 1H, J = 8 Hz, NH), 7.70 (d, 1H, J = 8 Hz, ArH of DNP), 7.59 (s, 1H, ArH of imidazole), 7.40 - 7.20 (m, 5H, PhH), 7.13 - 7.10 (br.m, 2H, NH), 7.04 (s, 1H, ArH of imidazole), 6.73 (d, 1H, J = 10 Hz, NH), 5.11 (d, 1H, J = 4 Hz, OH), 4.52 (dt, 1H, J = 6 Hz, □-H of His), 4.28 (dt, 1H, J = 5, 10 Hz, •-H of Phe), 4.19 (dd, 1H, J = 6, 7 Hz, □-H of Ile), 4.09 - 3.99 (br.m, 1H, C*H*OH), 3.94 - 3.80 (br.m, 1H, NC*H*CHOH), 3.66 (s, 3H, $OCH_3$), 3.36 - 2.88 (m, 6H, $CH_2$Ph, $CH_2$-imidazole and NC$H_2CH_2$), 2.58 - 2.25 (m, 2H, HOCHC$H_2$CO), 2.30 (t, 2H, J = 8 Hz, $CH_2CO_2$), 2.10 - 0.68 (m, 28H), 1.40 (s, 9H, $(CH_3)_3C$). MS (electrospray) 1006.8 ($M+H^+$) (1005.5 calcd. for $C_{50}H_{71}N_9O_{13}$).

Boc-Phe- His-ACHPA-Ile-6-AC-OMe (10).

To a solution of Boc-Phe-DNP-His-ACHPA-Ile-6-AC-OMe (9, 0.540 g, 0.537 mmol) in DMF (8 mL), thiophenol (1.35 mL, 13.2 mmol) is added. The mixture was stirred at 22°C overnight. The solution is concentrated in vacuo. The residue is applied to a silica gel column, which is then eluted first with $CH_2Cl_2$ to remove the relatively non-polar components, then with $CH_2Cl_2$ / MeOH / $NH_3$ (95 / 5 / 1). The positive fractions are pooled, concentrated in vacuo to give a brownish solid (10, 0.385 g) (yield: 85%). NMR spectra are: $^1$HNMR (300 MHz, $CD_3OD$) • 7.63 (s, 1H, ArH of imidazole), 7.32 - 7.17 (m, 5H, PhH), 6.93 (s, 1H, ArH of imidazole), 4.52 (t, 1H, J = 6 Hz, •-H of His), 4.30 (dd, 1H, J = 5, 10 Hz, •-H of Phe), 4.16 (d, 1H, J = 7 Hz, •-H of Ile), 4.00 - 3.86 (m, 2H, NC*H*C*H*OH), 3.65 (s, 3H, $OCH_3$), 3.27 - 3.00 and 2.89 - 2.78 (m, 6H, $CH_2$Ph, $CH_2$-imidazole and NC$H_2CH_2$), 2.42 - 2.21 (m, 2H, HOCHC$H_2$CO), 2.30 (t, 2H, J = 7 Hz, $CH_2CO_2$), 1.98 - 1.06 (m, 19H), 1.36 (s, 9H, $(CH_3)_3C$), 1.00 - 0.72 (m, 9H). MS (electrospray) 840.5 ($M+H^+$) (839.5

calcd. for $C_{44}H_{69}N_7O_9$).

Boc-Phe- His-ACHPA-Ile-6-AC-OH (11).

To a solution of Boc-Phe- His-ACHPA-Ile-6-AC-OMe (10, 0.180 g, 0.215 mmol) in THF (4 mL), 1N NaOH (1.0 mL) is added. The mixture is stirred at 22°C overnight. Water (10 mL) is added. THF is removed in vacuo to give a cloudy solution, which is acidified with 1NHCl to pH 2 to 3. The product is extracted with ethyl acetate three times. Each time, efforts are made to dissolve the light white precipitates (presumably the sodium salt of the free carboxylic acid). The combined ethyl acetate solutions are dried over $Na_2SO_4$, concentrated in vacuo to give a solid (11, 0.175 g) (yield: 99%). NMR spectra are: $^1$HNMR (300 MHz, $CD_3OD$) • 8.59 (s, 1H, ArH of imidazole), 8.20 (br.t, 0.5H, J = 5 Hz, NH), 7.78 (br.d, 0.5H, J = 8 Hz, NH), 7.37 - 7.12 (m, 5H, PhH), 7.30 (s, 1H, ArH of imidazole), 4.70 (dd, 1H, J = 6, 7 Hz, •-H of His), 4.28 (dd, 1H, J = 11, 5 Hz, •-H of Phe), 4.18 (d, 1H, J = 6 Hz, •-H of Ile), 4.03 - 3.90 (br.m, 2H, NCHCHOH), 3.42 - 3.02 and 2.89 - 2.75 (m, 6H, $CH_2Ph$, $CH_2$-imidazole and $NCH_2CH_2$), 2.43 - 2.30 (br.m, 2H, $HOCHCH_2CO$), 2.28 (t, 2H, J = 7 Hz, $CH_2CO_2$), 1.98 - 1.06 (m, 19H), 1.40 (s, 9H, $(CH_3)_3C$), 1.06 - 0.78 (m, 9H). MS (electrospray) 826.7 ($M+H^+$) (825.5 calcd. for $C_{43}H_{67}N_7O_9$).

Boc-Phe- His-ACHPA-Ile-6-AC- NHS (12).

To a mixture of P-EDC (555 mg, 0.63 meq / g, 350 • mol), Boc-Phe-His-ACHPA-Ile-6-AC-OH (11, 90 mg, 109 • mol) and NHS (25 mg, 217 • mol) in a sintered glass syringe tube, anhydrous DMF (6 mL) is added. After the substances are dissolved, $CHCl_3$ (6 mL) is added. The suspension is agitated by a stream of $N_2$ at 22°C overnight. The solution is filtered, and the resin was washed with DMF. The combined filtrates are concentrated in vacuo. The residue is purified by HPLC on a C18 column using a gradient of 10 - 70% $CH_3CN$ in $H_2O$ (containing 0.45% TFA) over 45 min. The positive fractions are pooled and lyophilized to afford a solid (12, 12 mg) (yield: 12%). NMR spectra are: $^1$H NMR (300 MHz, $CD_3OD$) • 8.83 (s, 1H, ArH of

imidazole), 8.17 (br.t, 0.5H, J = 6 Hz, NH), 7.68 (d, 0.5H, J = 8 Hz, NH), 7.40 (s, 1H, ArH of imidazole), 7.33 - 7.10 (m, 5H, PhH), 4.68 (t, 1H, J = 7 Hz, •-H of His), 4.27 (dd, 1H, J = 10, 6 Hz, •-H of Phe), 4.20 (d, 1H, J = 7 Hz, •-H of Ile), 4.04 - 3.90 (br.m, 2H, NCHCHOH), 3.40 - 3.00 and 2.95 - 2.77 (m, 6H, $CH_2Ph$, $CH_2$-imidazole and $NCH_2CH_2$), 2.83 (s, 4H, $OCCH_2CH_2CO$), 2.62 (t, 2H, J = 7 Hz, $CH_2CO_2N$), 2.30 (d, 2H, J = 6.5 Hz, $HOCHCH_2CO$), 1.96 - 1.08 (m, 19H), 1.38 (s, 9H, $(CH_3)_3C$), 1.08 - 0.77 (m, 9H). MS (electrospray) 923.7 ($M+H^+$) (922.5 calcd. for $C_{47}H_{70}N_8O_{11}$).

Boc-Phe-His-ACHPA-Ile-6-AC-Salicyl t-Butyl Ester (14).

A solution of salicyl t-butyl ester (13, 24 mg, 0.125 mmol) and potassium t-butoxide (14 mg, 0.125 mmol) in anhydrous THF (1.0 ml) is quickly mixed with solid Boc-Phe- His-ACHPA-Ile-6-AC- NHS ester (12, 91 mg, 0.098 mmol). The solution is then stirred at 22° for 20 min. The solvent is removed in vacuo, water is then added, and the solution is acidified with acidic acid to pH 4-5. The precipitates are collected by vacuum filtration, then dissolved in MeOH. The solution is washed with hexane three times to remove excess of salicylic t-butyl ester and then concentrated in vacuo to give a solid (36 mg), which is purified by HPLC using a gradient of $CH_3CN$ in $H_2O$ as eluents varying from 10 to 100% over 55 min. The positive fractions are pooled and lyophilized to give a powder (14, 7.9 mg) (representative yield 8%). MS (electrospray) 1003.2 (M+H+) 1002.2 calculated for $C_{54}H_{79}N_7O_{11}$.

Boc-Phe- His-ACHPA-Ile-6-AC-Salicylate (15).

Compound (15, 1.3 mg) is dissolved in TFA (1ml) at room temperature. The solution is allowed to stand at room temperature for 2 hours. TFA was removed in vacuo to give a solid (1.0 mg). MS (electrospray) for $C_{50}$ $H_{71}N_7O_{11}$. MS (electrospray) 1003.2 (M+H+) 1002.2 calculated for $C_{50}H_{71}N_7O_{11}$.

## Example VI

**Synthesis of a calcium channel blocker-salicylate derivative**

The synthesis scheme is illustrated in Fig. 6.

3,5-Pyridinedicarboxylic acid-2-[N-adipoyl-2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-(3-ethyl ester-5-methyl ester)(3)

To a solution of 3,5-pyridinedicarboxylic acid-2-[2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-(3-ethyl ester-5-methyl ester, 1, amlopidine)) (165.6 mg, 0.405 mmol) in absolute $CH_2Cl_2$ (6 ml), adipic anhydride 2, 77.9 mg, 0.608 mmol) (prepared according to E.H. Farmer, J. Kracovski, J. Chem. Soc., 680 (1927)), and 4-(dimethylamino)pyridine (0.2 mg) were added. The turbid mixture is stirred for 21 hours and then evaporated, the resulting oil dissolved in etyl acetate, and the solution extracted with water and 5 times with saturated NaHCO3 solution. Acidificiation of the sodium bicarbonate phase with 2N $H_2SO_4$, extraction with ethyl acetate, drying and evaporation under high vacuum afforded an oil 3. (134 mg, 0.251 mmol, 62% yield) MS (electrospray) ave 538.1 (M+H+) (ave 537.0 calculated for $C_{26}H_{33}N_2O_8Cl$).

3,5-Pyridinedicarboxylic acid-2-[N-adipoyl-2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-(3-ethyl ester-5-methyl ester) NHS-ester(5)

To a mixture of P-EDC (1.11 g, 0.7 mmol), 3,5-Pyridinedicarboxylic acid-2-[N-adipoyl-2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-(3-ethyl ester-5-methyl ester)(3) (134 mg, 0.251 mmol) and NHS (58 mg, 0.50 mmol) in a sintered glass syringe tube, anhydrous DMF is added (12 ml). After the substances are dissolved, $CHCl_3$ is added. The suspension is agitated by a stream of $N_2$ at 22°C overnight. The solution is filtered, and the resin is washed with DMF. The combined filtrates are concentrated in vacuo. The residue is purified by HPLC on a C18 column using a gradient of 10-70% $CH_3CN$ in $H_2O$ (containing 0.45% TFA) over 45 min. The positive fractions

are pooled and lyophilized to afford a solid (5) 77.3 mg, 48% yield.

3,5-Pyridinedicarboxylic acid-2-[N-adipoyl-2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-(3-ethyl ester-5-methyl ester)-Salicylic acid(6)

A solution of salicyl t-butyl ester (13, 24 mg, 0.125 mmol) and potassium t-butoxide (14 mg, 0.125 mmol) in anhydrous THF (1.0 ml) is quickly mixed with 3,5-pyridinedicarboxylic acid-2-[N-adipoyl-2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-(3-ethyl ester-5-methyl ester)NHS-ester (5). The solution is then stirred at 22° for 20 min. The solvent is removed in vacuo, water is then added, and the solution is acidified with acetic acid to pH 4-5. The precipitates are collected by vacuum filtration, then dissolved in MeOH. The solution is washed with hexane three times to remove excess of salicylic t-butyl ester and then concentrated in vacuo to give a solid (36 mg), which is purified by HPLC using a gradient of $CH_3CN$ in $H_20$ as eluants varying from 10 to 100% over 55 min. The positive fractions are pooled and lyophilized to give a powder. The powder is dissolved in TFA (1 ml) at room temperature. The solution is allowed to stand at room temperature for 2 hours. TFA is removed in vacuo to give a solid (6) MS (electrospray) ave 658.2 (M+M+) (ave 657.1 for $C_{33}H_{37}N_2O_{10}Cl$) (1.6 mg, 2% yield).

## EXAMPLE VII

**Anti-thrombin activity of HSA labeled with Argatroban Salicylates compounds of the present invention**

Human serum albumin (HSA, Sigma Cat. # A-8763) at a concentration of 300 µg/ml (= 4.5 µM) was reacted with 100 µM arg-C6 SAL (YS-41-13), arg-C12 SAL (QC-37-49P) or arg-C18 SAL (YS-41-94 as shown on Fig.9) in PBS, pH 7.4 for 4 hours at 37°C (or 3 hours at 42°C for argC12-sal).

Samples were purified over 5 ml Dextran™ column equilibrated in PBS. Seven fractions of 1 ml were collected and protein concentration was determined with BCA kit (bicinchoninic acid, Pierce). Fractions 2-4 (containing conjugate) were pooled and concentrated using Centricon 30™ (Amicon). Protein concentration was checked again after concentration with BCA kit. Conjugates were stored at -80°C until use.

Thrombin from human plasma was obtained from Enzyme System Products, Dublin, California, in glycerol:water 1:1, 11.4 mg/ml, 3800 U/mg, MW 36,700, and stored undiluted at -20°C. It was freshly diluted the day of use.

To dilute for the assay, 0.67 ml (25 Units) of thrombin was pipetted into 5 ml of 0.1 mg/ml BSA (from Pierce, Catalog No. 23209, BSA fraction V, a 2 mg/ml stock in saline with sodium azide), in cold PBS (GIBCO, Catalog No. 14190-136, without calcium chloride and magnesium chloride, pH 7.2) and briefly vortexed, yielding a stock of 5 mU thrombin/ml. A second dilution of 250 ml of this stock into 9.75 ml of 0.1 mg BSA/ml PBS resulted in a 0.125 mU/ml, or 3.125 mU/25ml, final working stock.

The substrate was N-tBOC-b-Benzyl-Asp-Pro-Arg-7-Amido-4-Methylcoumarin HCl and was obtained from Sigma, Catalog No. B-4028, Lot 122H0070, F.W. 770.3. Five mg was dissolved in 1.42 ml DMSO for a 4 mM stock. The stock was stored in 200 ml aliquots at -20°C in the dark.

An alternative substrate was N-tBOC-Val-Pro-Arg-7-Amido-4-Methylcoumarin HCl and was obtained from Sigma, Catalog No. B-9385, Lot 53H0805, F.W. of free base 627.7. Five mg was dissolved in 1.863 ml DMSO for a 4 mM stock. The stock was stored in 200 ml aliquots at -20°C in the dark.

The fluorescent standard was 7-Amido-4-Methylcoumarin and was obtained from Aldrich, Catalog No. 25,737-0, MW 175.19. 6.1 mg was dissolved in 1.743 ml of DMSO for a 20 mM stock. The stock was stored in 200 ml aliquots at -20°C in the dark.

Assays were performed kinetically at ambient temperature (about 20°C) in 96-well microtiter plates (Nunc Immunomodule Maxisorb, a flat

bottomed polystyrene plate) on a Perseptive Biosystems Cytofluor I™. Stock solutions containing common concentrations and components for each well or group of well were prepared and 200 ml aliquotted per microtiter well. Assays were routinely conducted in a total volume of 250 ml, which allows for 25 ml for inhibitor and 25 ml for thrombin (about 3 mU).

For an initial determination of Km and Vmax, the concentration of the fluorogenic substrate N-tBOC-b-Benzyl-Asp-Pro-Arg-7-Amido-4-Methylcoumarin was varied from 5 to 200 mM.

For inhibition assays, final concentrations per well (250 ml VT) included 25 mM N-tBOC-b-Benzyl-Asp-Pro-Arg-7-Amido-4-Methylcoumarin HCl (approximately Km), 0.1 mg BSA/ml PBS, and 1% DMSO. Initial readings were taken to ensure exclusion of rogue wells with abnormally high backgrounds (I excitation at 360 nM, bandwidth 40 nM, I emission, 460 nm, bandwidth 40 nm). Then, 25 ml of a 10X stock of the putative inhibitor were added per well, mixed for 5 seconds and read kinetically for 10 minutes at ambient temperature to assess background interference and/or any increase in hydrolysis of substrate due to inhibitor.

In inhibition assays using argatroban or soluble, tethered variants of argatroban (YS-41-13, QC-37-48 and YS-41-94), samples were dissolved in DMSO at 1 mM, then diluted appropriately for assay in 1% DMSO in the buffer specified above.

The thrombin assay was initiated by adding 25 ml of thrombin/well with a multichannel pipettor and mixed for 5 seconds. The appearance of the fluorescent product (free 7-amido-4-methylcoumarin released from the peptide by thrombin) was followed simultaneously in 48 wells (one reading per minute for 30 minutes, I excitation at 360 nm, bandwidth 40 nm, I emission, 460 nm, bandwidth 40 nm). The velocity of the reaction was determined as an initial velocity in the first 10 minutes, with the signal in relative fluorescent units (RFU) of an uninhibited reaction about 9,000-10,000, above an initial background reading of about 1,000 (gain setting of 80). Routinely, less than 5% of the initial substrate was converted to fluorescent product during 10 minutes, and samples and curves are run in duplicate. A standard curve of

thrombin activity from 0.3125-3.5 mU/well was included, as well as a standard curve of 7-amido-4-methylcoumarin (ranging from 5 to 200 pmoles/well in 1% DMSO, 0.1 mg BSA/ml PBS, VT 250 ml/well). The pH of each sample was verified as 7.2 with pH paper at the end of the assay.

The results of the different argatroban-salicylates compounds of the present invention are as follows:

| has labeled with | Arg-C6-SAL | Arg-C12-SAL | Arg-C18-SAL |
|---|---|---|---|
| IC50 | 8μM | 4μM | 1μM |

## EXAMPLE VIII

### LC-Biotin 5-Bromo Salicylate (QC-28-91) Labels HSA Specifically in Whole blood (Fig.10)

Human blood collected on EDTA was treated with the salicylate (QC-28-91, SAL) at 100 μM final concentration, along with other non-specific biotinylating reagents (100 μM), including biotin-NHS, biotin-maleimide, biotin ALL5 (molecule known for its specificity of bonding onto HSA), and a negative control with DMSO, at room temperature under constant agitation for 1 hour. Blood was centrifuged 5 min. at 2500 rpm to obtain plasma and blood cells. Ten μl of plasma diluted 1/10 in PBS were separated by SDS-PAGE (8%) under non reducing and reducing conditions (with 10 mM DTT). Then, plasma proteins were transferred onto nitrocellulose sheet for 1 hour at 2 $mA/cm^2$ using a semi-dry transfer apparatus. Nitrocellulose was saturated for 2 hours at 37°C with TBS (10 mM tris, 150 mM NaCl, pH 7.4) containing 5% gelatin and 0.1% Tween™-20. After 3 washes with TBS, 0.1% Tween™-20 for 10 min., blot was incubated with avidin-HRP (Sigma) diluted 1/200 000 in TBS, 1% gelatin, 0.1% Tween™-20 for 30 min. at RT. After 3 washes as above, detection was performed using ECL method (Amersham). The results are illustrated in Fig. 10. It was found that the salicylate derivative specifically labels Human Serum Albumin, as detected under reduced conditions (10 mM DTT) as shown on the Western blot. While biotin-NHS and biotin-MAL also label many other blood components, the salicylate only labels HSA. It was

also found that the salicylate does not biotinylate RBC membranes, while the biotin-NHS and biotin-MAL do.

## EXAMPLE IX

**Mass Spectrometric Analysis of Tryptic Digest of HSA Labeled with Arg-C6 5-Bromo Salicylic Acid (YS-41-13): Lysine 199 is the Labeling Site (Fig. 11)**

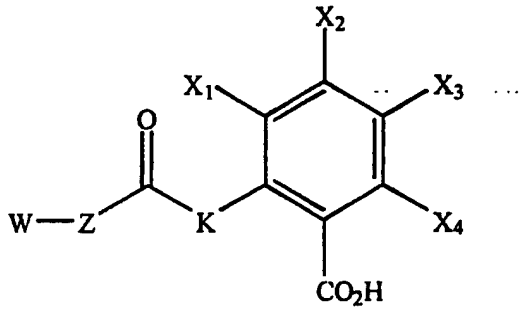
Human serum albumin (4.5 mM) was incubated with Arg-C6 salicylate (YS-41-13) (100 mM) in PBS (1.00 ml) at 37°C for 1 hour. The labeled HSA was purified by a Dextran Pierce KwikSep™ column (5 ml), which was eluted with 200 mM Tris HCl buffer (pH 8.5, 5 column volumes). Positive fractions were pooled, 0.900 ml of which was taken and treated with 8 M guanidinium HCl, 1.3 mM EDTA and 1 M DTT. The solution was flushed with argon, then heated at 60°C for 45 min. After being cooled to room temperature, iodoacetate (250 mg/ml, 5 ml) was added. The solution was flushed with argon, and allowed to stand at room temperature in dark for 30 min.. It was dialyzed immediately with microdialyzer into 50 mM ammonium bicarbonate (pH 8.0) overnight. The dialyzed sample was then subject to trypsin digestion (2% trypsin) at 37°C for 4 hours. A control sample was obtained from HSA which was treated the same way as described above except it was not treated with the salicylate.

Both samples were analyzed by HPLC/MS. As shown in panel A of Fig. 11, for the control sample there is a peak at 44.6 min. which corresponds to the peptide fragment 198-205. However, in the treated sample, this peak has disappeared, instead a new peak at 77.9 min. appeared (as shown in panel C of Fig. 11), which was absent in the control sample (panel A). Mass measurement of the peak at 77.9 min. gave a value of 776.9, corresponding to the peptide fragment 198-205 plus an argatroban molecule (LK*BASLQK; where K*=K+Arg, B=carboxymethyl cysteine). The experiment established that lysine 199 is the residue which was argatrobanylated.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

**WHAT IS CLAIMED IS:**

1. A salicylate compound which comprises a compound of the formula:




wherein:

$X_1$, $X_2$, $X_3$ and $X_4$ are selected from the group consisting of H, Cl, Br, I, F, $NO_2$, CN, Me, $CO_2$ or MeO,

Z is a spacing group which links W and wherein said linked W remains bioavailable and bioactive;

W is a therapeutic or diagnostic agent; and

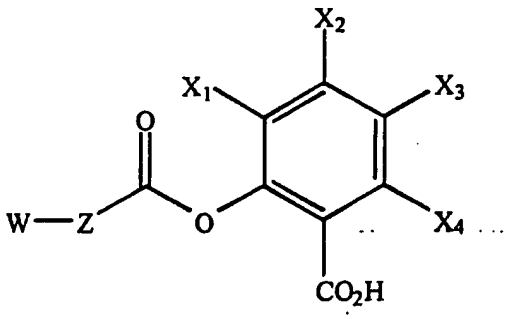
K is O, N or S.

2. The compound of according to claim 1, wherein Z is of at least 6 atoms.

3. The compound of according to claim 2, wherein Z is selected from the group consisting of alkyl, alkoxy, alkenyl, alkynyl, amino, alkyl-substituted amino groups, cycloalkyl, polycyclic, aryl, polyaryl, substituted aryl heterocyclic and substituted heterocyclic groups.

4. The compound of according to claim 1, wherein W is a drug or a small organic molecule of molecular weight < 2000.

5. A salicylate compound for specific covalent labeling of albumin at Lys 199, which comprises a compound of the formula:



wherein:

$X_2$ and $X_4$ are H;

$X_1$ and $X_3$ are selected from the group consisting of Cl, Br, I, F, $NO_2$, CN, Me or MeO;

Z is a hydrophilic spacing group which links W and wherein said linked W remains bioavailable and bioactive; and

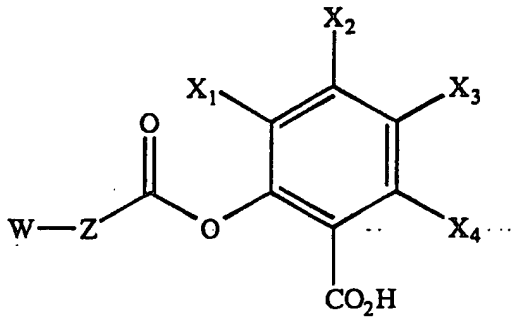
W is a biologically active molecule.

6. The compound of according to claim 5, wherein Z is of at least 6 atoms.

7. The compound of according to claim 6, wherein Z is selected from the group consisting of alkyl, alkoxy, alkenyl, alkynyl, amino, alkyl-substituted amino groups, cycloalkyl, polycyclic, aryl, polyaryl, substituted aryl heterocyclic and substituted heterocyclic groups.

8. The compound of according to claim 5, wherein W is a drug or a small organic molecule of molecular weight < 2000.

9. A salicylate compound for specific covalent labeling of albumin at Lys 199, which comprises a compound of the formula:



wherein:

$X_1$, $X_2$ and $X_4$ are H;

$X_3$ is Br;

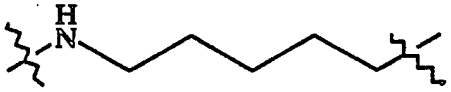
Z is a hydrophilic spacing group which links W and wherein said linked W remains bioavailable and bioactive; and

W is a biologically active molecule.

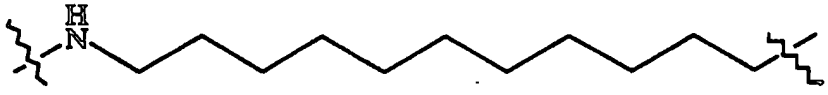
10. The compound of according to claim 9, wherein Z is of at least 6 atoms.

11. The compound of according to claim 10, wherein Z is selected from the group consisting of alkyl, alkoxy, alkenyl, alkynyl, amino, alkyl-substituted amino groups, cycloalkyl, polycyclic, aryl, polyaryl, substituted aryl heterocyclic and substituted heterocyclic groups.

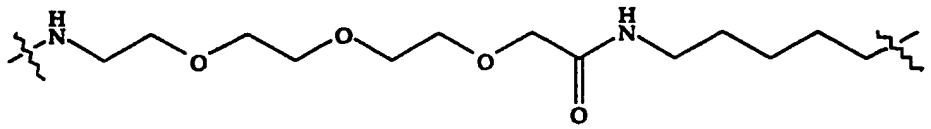
12. The compound of according to claim 11, wherein Z has the formula:



13. The compound of according to claim 11, wherein Z has the formula:



14. The compound of according to claim 11, wherein Z has the formula:



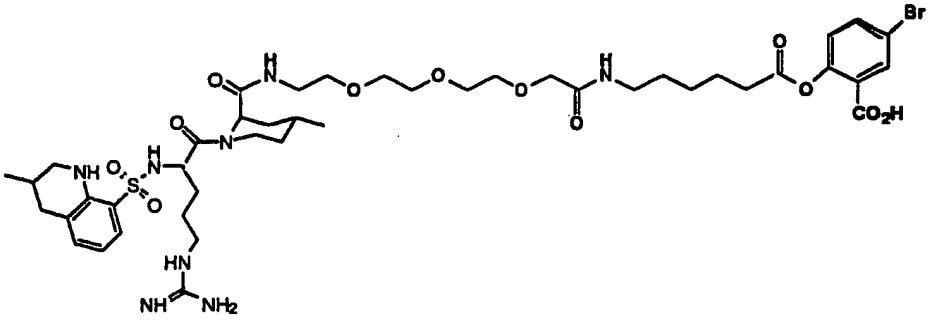
15. The compound of according to claim 9, wherein W is a drug or a small organic molecule of molecular weight < 2000.

16. The compound of according to claim 9, wherein W is a drug of molecular weight < 1000.

17. The compound of according to claim 9, wherein W is biotin.

18. The compound of according to claim 9, wherein W is argatroban.

19. A salicylate compound for specific covalent labeling of albumin at Lys 199, which comprises a compound of the formula:



20. A method for the *in vivo* specific labeling of albumin at Lys 199 with a biologically active molecule, which comprises:

introducing into the bloodstream of a mammalian host a salicylate compound of claim 1 in an amount sufficient to provide an effective amount of said biologically active molecule; wherein said compound reacts with and becomes covalently bound to serum albumin at Lys 199 and said biologically active molecule remains bioavailable and bioactive over an extended period of time as compared to the lifetime of unbound biologically active molecule.

21. The method of claim 20, wherein said biologically active molecule is introduced intravascularly.

22. A blood portion comprising a compound according to claim 1 covalently and specifically bound to at least one serum albumin at Lys 199.

23. A conjugate comprising a compound according to claim 1 covalently and specifically bound to serum albumin at Lys 199.

24. A method for the *in vitro* specific labeling of albumin at Lys 199 with a biologically active molecule, which comprises:

introducing into an *in vitro* solution selected from the group consisting of blood, plasma, serum, and saline buffer, a salicylate compound of claim 1 in an amount sufficient to provide an effective amount of said biologically active molecule; wherein said compound reacts with and becomes covalently bound to serum albumin at Lys 199 to form a biologically active molecule-albumin conjugate, wherein said biologically active molecule-albumin conjugate is suitable for injection into the bloodstream of a mammalian host and said biologically active molecule remains bioavailable and bioactive over an extended period of time as compared to the lifetime of unbound biologically active molecule.

25. The method of claim 24, wherein said *in vitro* solution is serum or saline buffer.

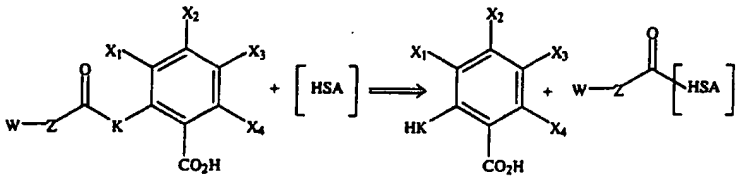



FIGURE 1

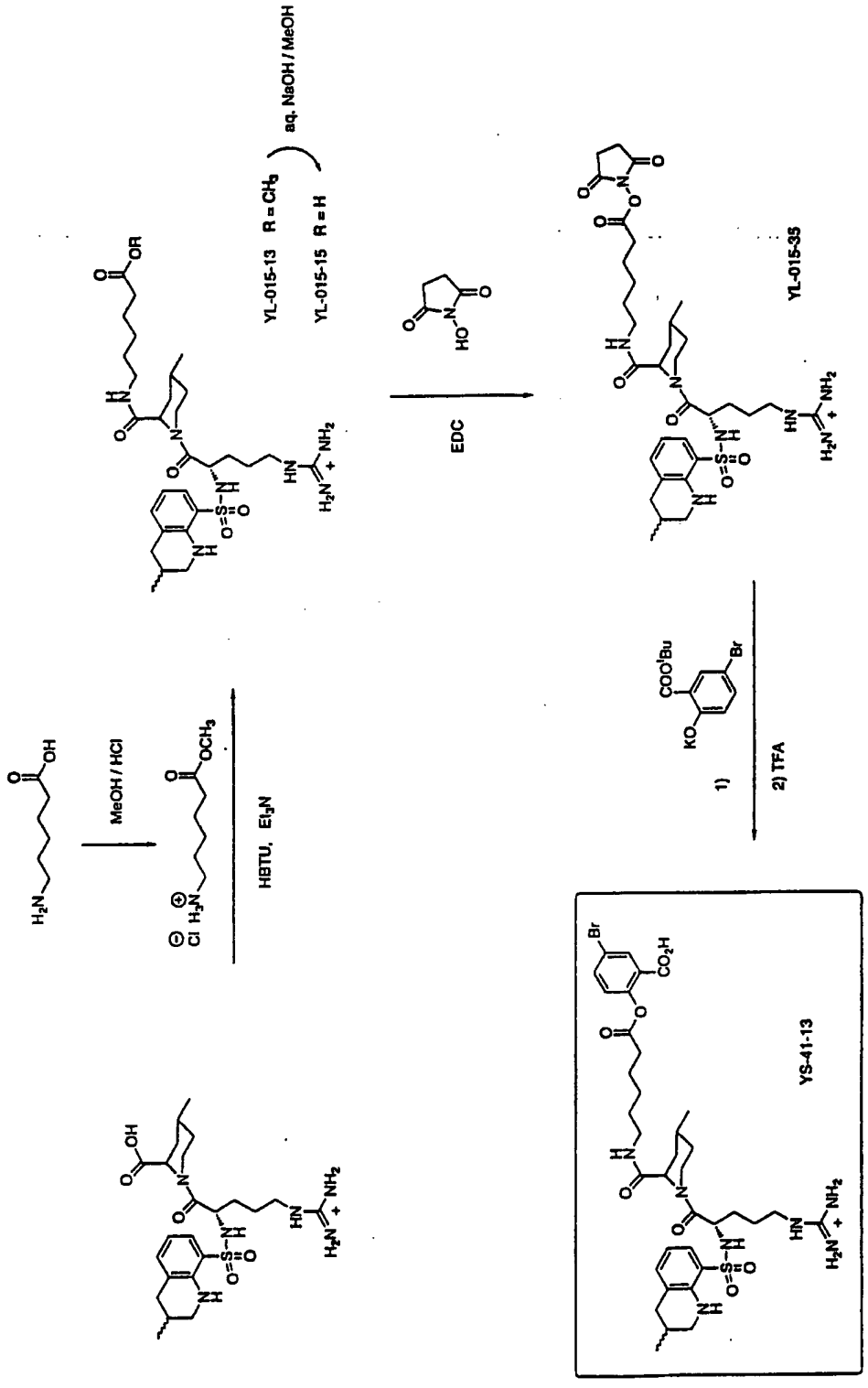

MeOH / HCl
HBTU, Et3N
aq. NaOH / MeOH
YL-015-13 R = CH3
YL-015-15 R = H
EDC
1)
2) TFA
YL-015-35
YS-41-13

FIGURE 2

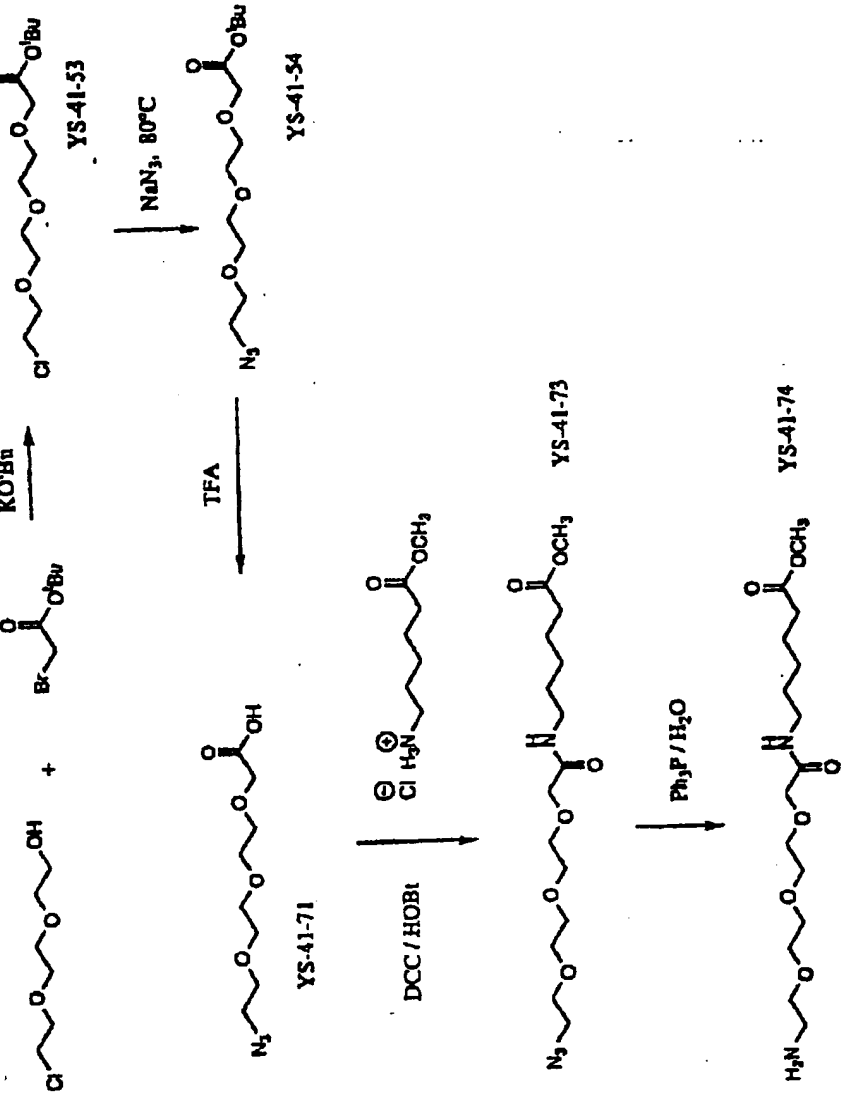

KO'Bu
YS-41-53
NaN3, 80°C
YS-41-54
TFA
YS-41-71
DCC / HOBt
YS-41-73
Ph3P / H2O
YS-41-74

FIGURE 3A

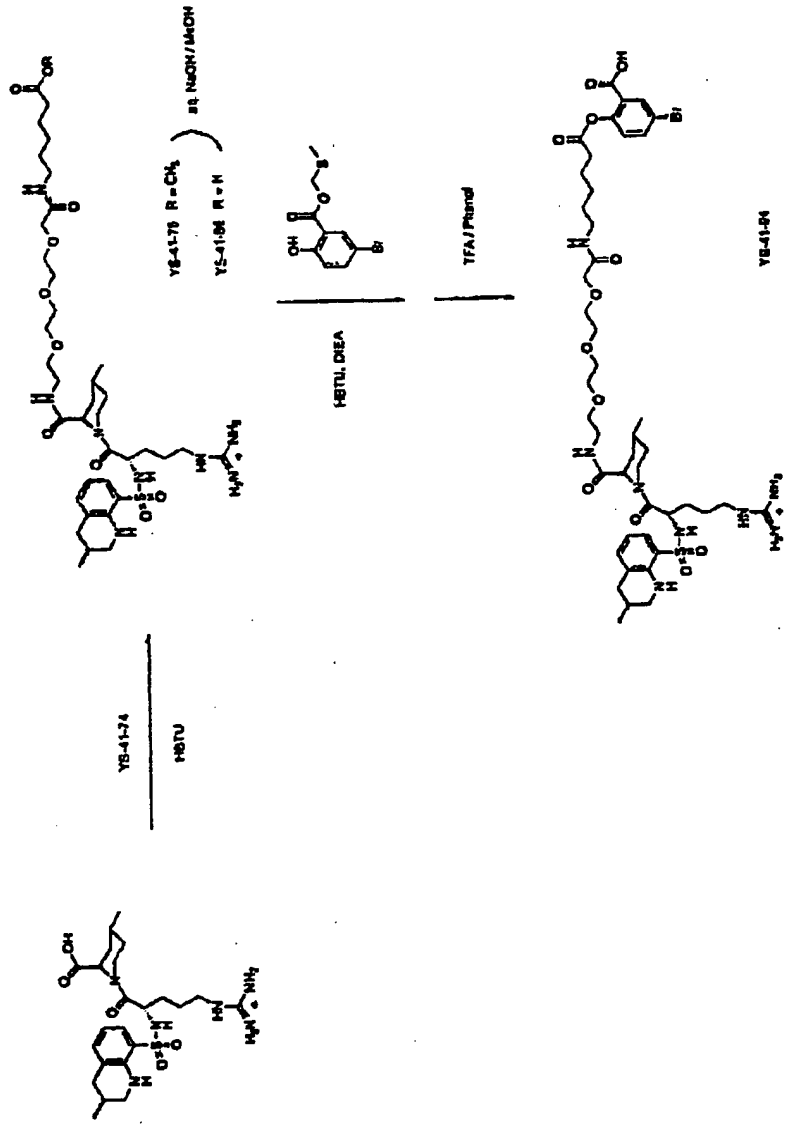

YS-41-79 R=CH3
YS-41-86 R=H
aq NaOH/MeOH
HBTU, DIEA
TFA / Phenol
YS-41-84
YS-41-74
HBTU

FIGURE 3B

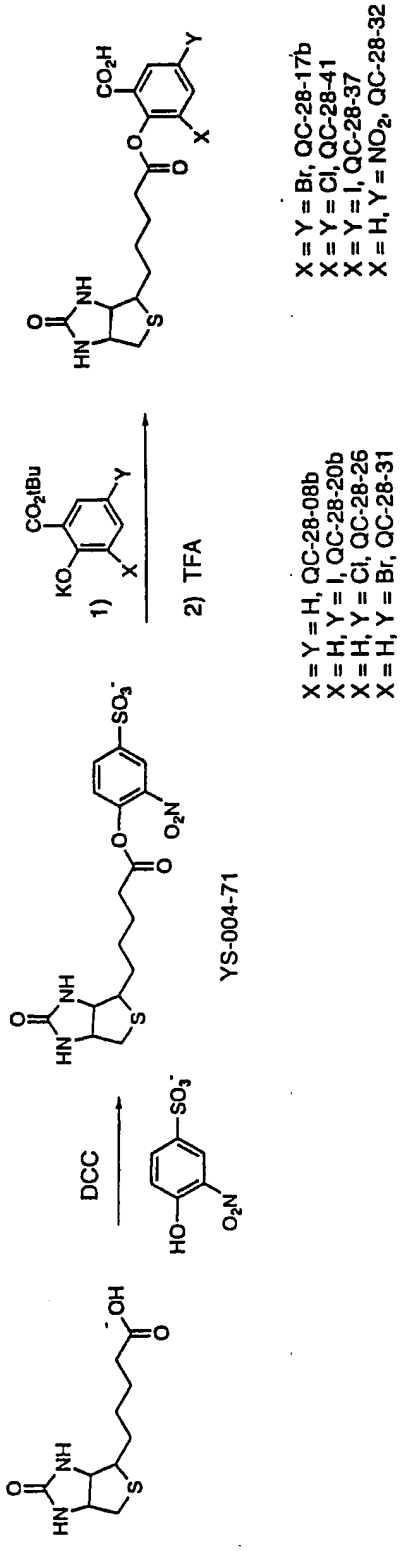

tBuOH
DCC
tBuOK
DCC
1)
2) TFA
X = Y = H, QC-28-08b
X = H, Y = I, QC-28-20b
X = H, Y = Cl, QC-28-26
X = H, Y = Br, QC-28-31
X = Y = Br, QC-28-17b
X = Y = Cl, QC-28-41
X = Y = I, QC-28-37
X = H, Y = NO2, QC-28-32
YS-004-71

FIGURE 4

Scheme 1

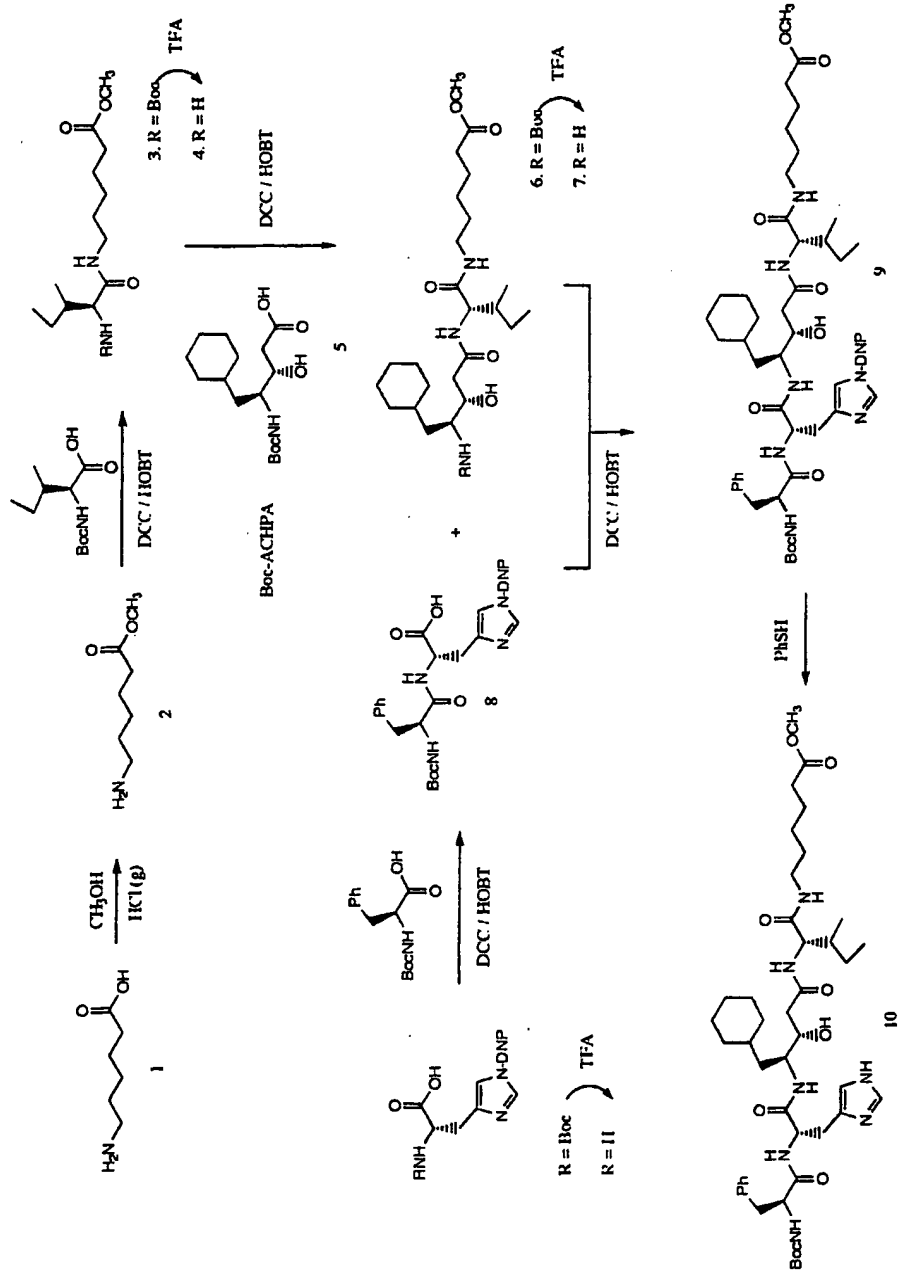



FIG. 5A

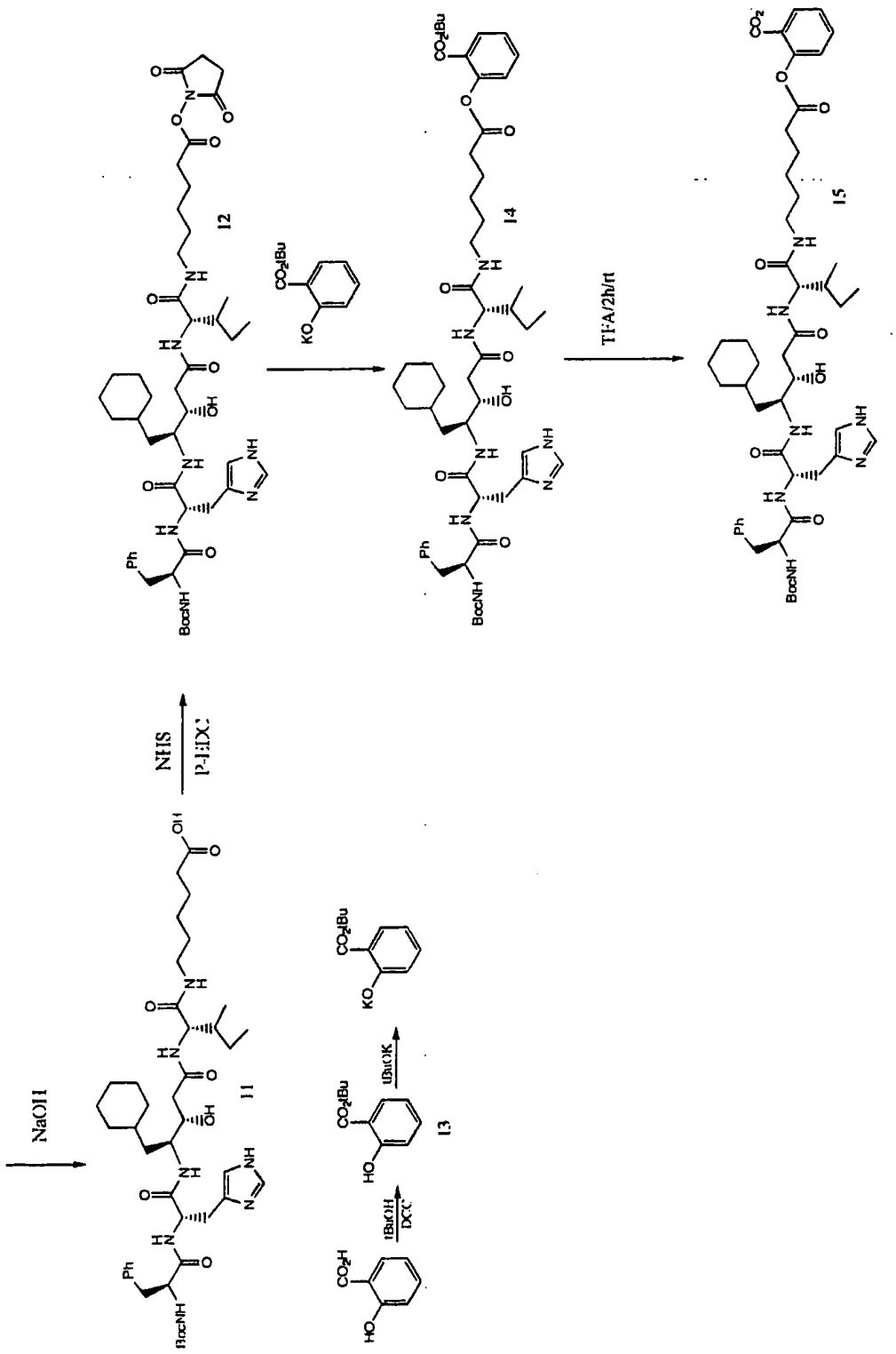

NaOH
11
NHS
DCC
12
tBuOH
DCC
13
tBuOK
14
TFA/2h/rt
15
BocNH
Ph
NH
OH
O
HO
KO
CO2tBu
CO2H

FIGURE 5B

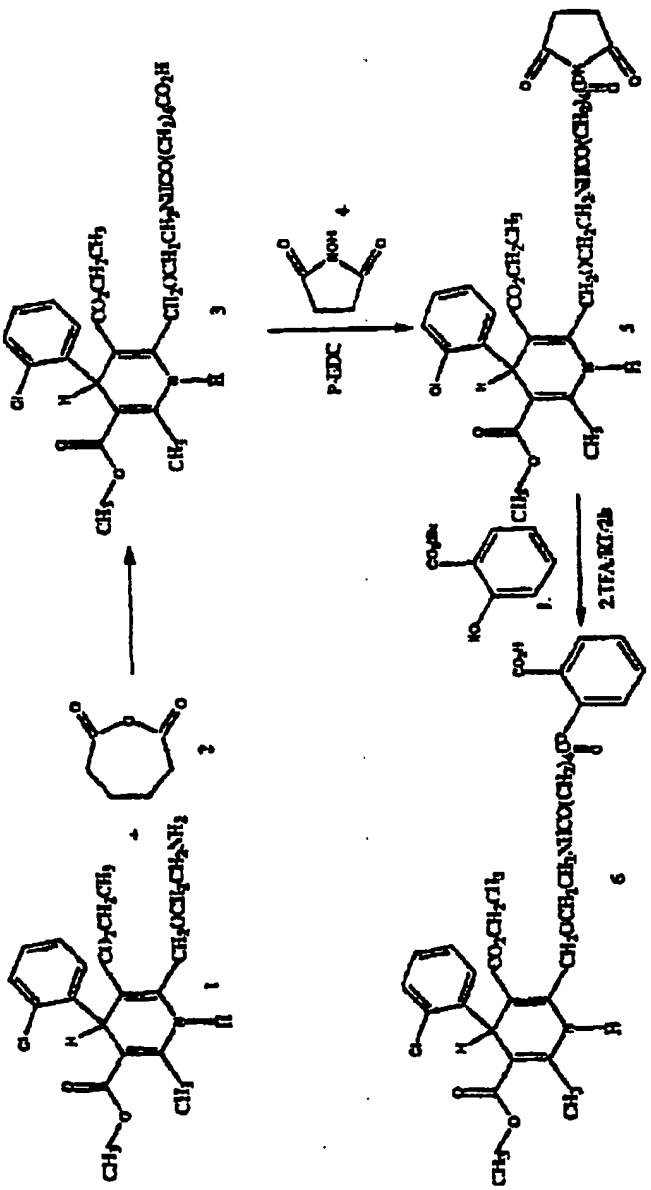
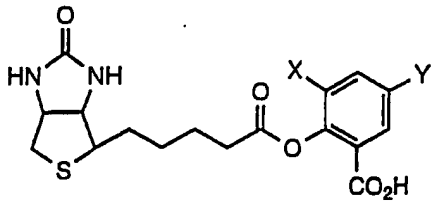
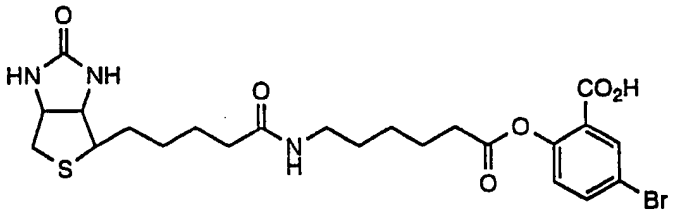


FIGURE 6



| Structure | Identification | Yield |
|---|---|---|
| X = Y = H | QC-28-8b | 64% |
| X = H, Y = I | QC-28-20b | 74% |
| X = H, Y = Cl | QC-28-26 | 72% |
| X = H, Y = Br | QC-28-31 | 52% |
| X = Y = Br | QC-28-17b | 77% |
| X = Y = Cl | QC-28-41 | 84% |
| X = Y = I | QC-28-37 | 81% |
| X = H, Y = $NO_2$ | QC-28-32 | 12% |
| X = H, Y = $CH_3$ | QC-28-63 | 9% |
| X = H, Y = F | QC-28-80 | 60% |
| X = H, Y = $OCH_3$ | QC-28-76 | 75% |



QC-28-91 14% Yield NMR LC-MS

FIGURE 7

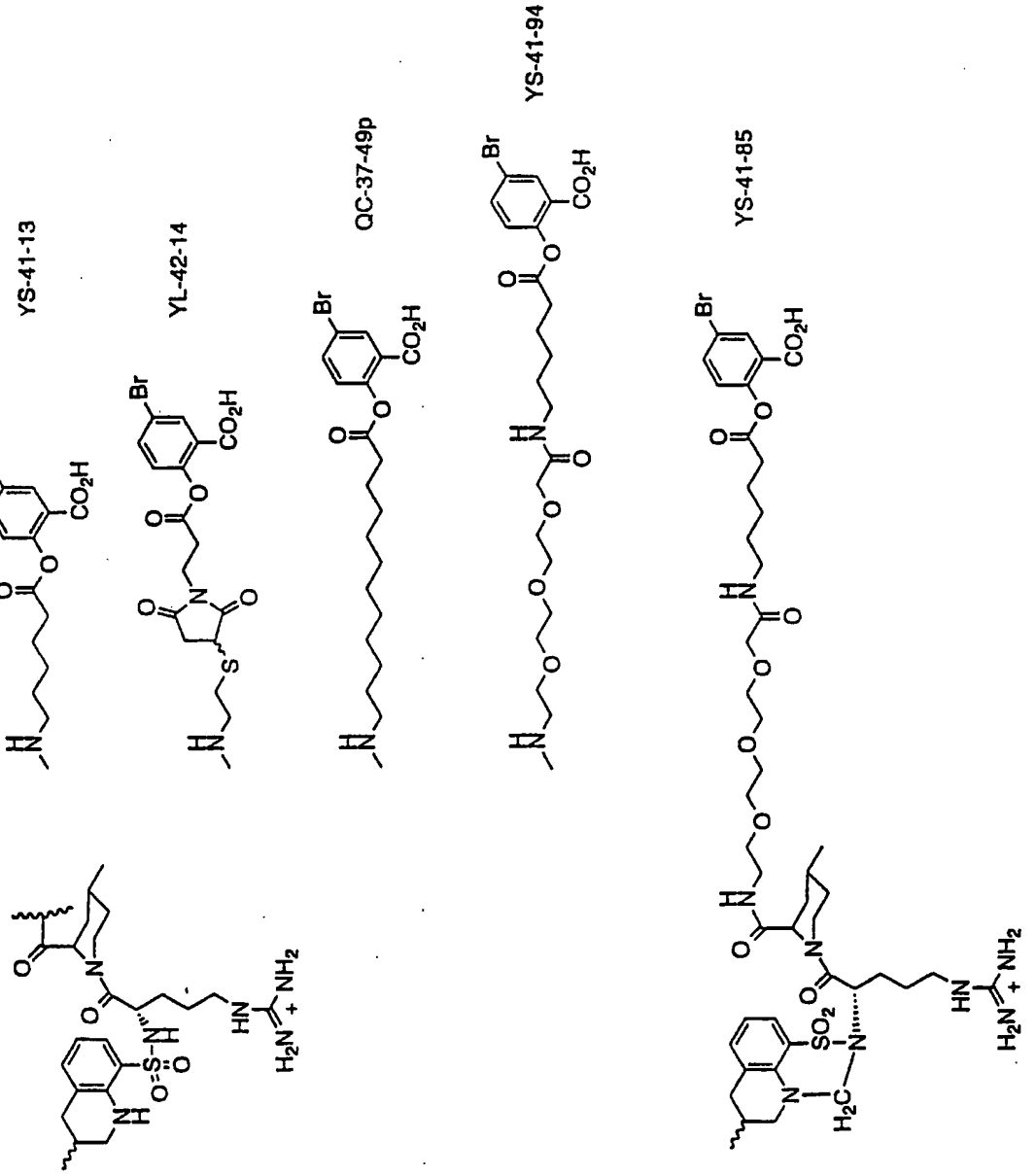



FIGURE 8

THROMBIN INHIBITION BY YS-41-94 AFTER CONJUGATION TO HSA

FIGURE 9

• Whole blood treated with 100µM of "Labeling entities"

• Western bloting (gel 8%), detection with peroxidase-labeled Streptavidin

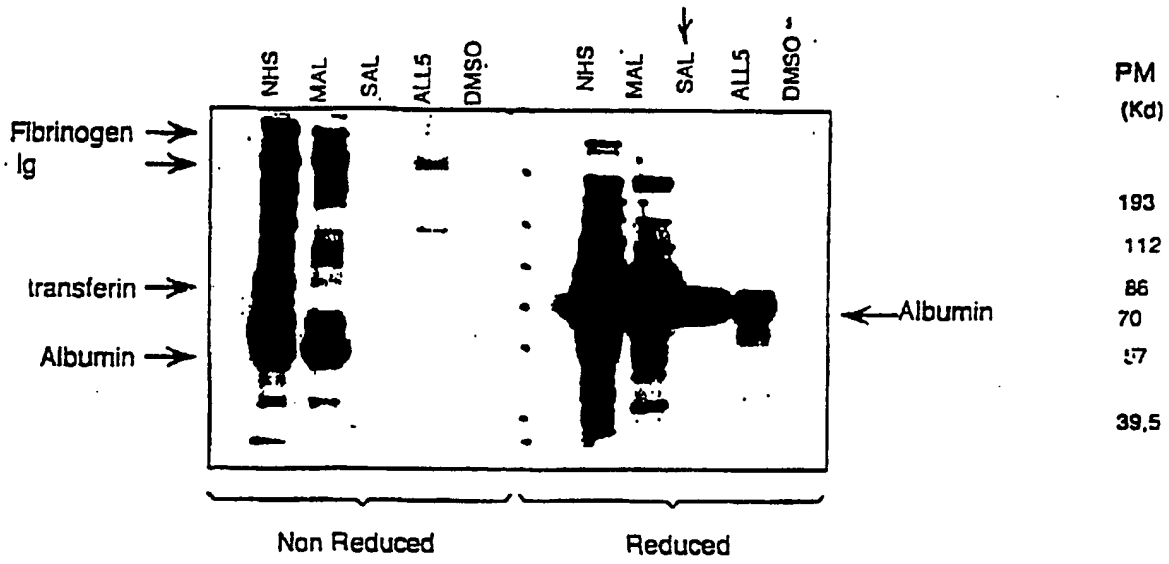



FIGURE 10

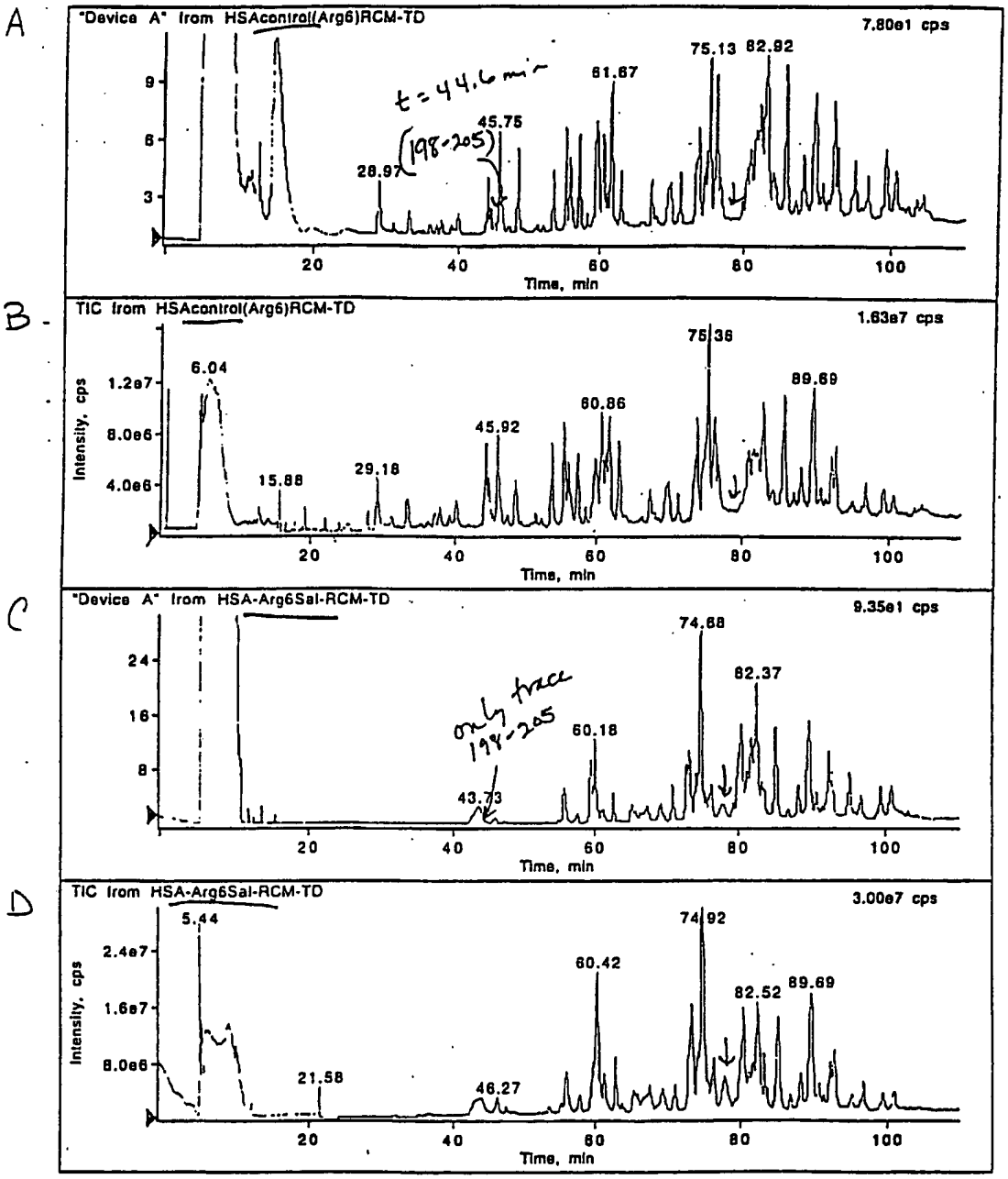

A
"Device A" from HSAcontrol(Arg6)RCM-TD
7.80e1 cps
t = 44.6 min
(198-205)
28.97
45.75
61.67
75.13
82.82
Time, min
B
TIC from HSAcontrol(Arg6)RCM-TD
1.63e7 cps
Intensity, cps
6.04
15.88
29.18
45.92
60.86
75.38
89.69
Time, min
C
"Device A" from HSA-Arg6Sal-RCM-TD
9.35e1 cps
only trace
198-205
43.73
60.18
74.68
82.37
Time, min
D
TIC from HSA-Arg6Sal-RCM-TD
3.00e7 cps
Intensity, cps
5.44
21.58
46.27
60.42
74.92
82.52
89.69
Time, min

FIGURE 11

SEQUENCE LISTING

<110> KRANTZ, ALEXANDER
SONG, YONG HONG
BRIDON, DOMINIQUE
HARDY, MARGARET
CHEN, QUI QUI
SETTINERI, TINA

<120> SPECIFIC COVALENT LABELING OF SERUM ALBUMINS WITH SALICYLATE DERIVATIZED THERAPEUTIC AND DIAGNOSTIC AGENTS

<130> REDC-900

<150> US 60/064,705

<151> 1997-11-07

<160> 5

<170> PC-DOS/MS-DOS

<210> 1

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<221>

<222>

<223> Dynorphin analogue (A 1-17).

<400> 1

Tyr Gly Gly Phe Leu Arg Arg Ile Arg Pro
1 5 10

Lys Leu Lys Trp Asp Asn Gln
15

<210> 2

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<221>

<222>

<223> Dynorphin analogue (A1-13)

<400> 2

Tyr Gly Gly Phe Leu Arg Arg Ile Arg Pro Lys Leu Lys
1 5 10

<210> 3

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<221>

<222>

<223> RGD containing peptide sequence

<400> 3

Arg Ile Ala Arg Gly Asp Phe Pro Asp Asp Arg Lys
1 5 10

<210> 4

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<221>

<222>

<223> KGD containing peptide sequence

<400> 4

Cys Arg Val Ala Lys Gly Asp Trp Asn Asp Asp Thr Cys
1 5 10

<210> 5

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<221>

<222>

<223> cyclic RGD containing peptide sequence.

<400> 5

Cys Arg Ile Ala Arg Gly Asp Phe Pro Asp Asp Arg Cys
1 5 10